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Poster

P047. Autism: Physiology and Systems III

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P047.01

Topic: A.07. Developmental Disorders

Support: SURP to Sandy Cheng
CfR to JW Lee

Title: Decreased neurogenesis and abnormal development of ventricular zone and lateral ganglionic eminence in the embryonic brain of BTBR T+ tf/J mice: correlation between embryonic neuroanatomy and idiopathic autism spectrum disorder

Authors: *J. LEE, S. CHENG;
Dept. of Biol., William Paterson Univ., Wayne, NJ

Abstract: Autism spectrum disorder (ASD) is a developmental disorder, where genetic abnormalities and exposure to environmental agents during embryonic development have been suggested as possible causes. Using BTBR T+ tf/J (BTBR) mouse, an animal model for idiopathic ASD, we examined the pattern of neocortex development that may be associated with expression of ASD-like behaviors. In first experiment, to examine the pattern and location of embryonic neurogenesis, EdU (50mg/kg, IP) was injected to timed-pregnant BTBR and C57 dams, and embryos (E16 and E18) were removed 12hrs later. Brains were frozen-sectioned and processed for Click reaction. In second experiment, to examine the development and rate of maturation of cortical radial glial and intermediate progenitor cells, embryonic brains (E16 and E18) of BTBR and C57 mice were frozen-sectioned and double-labeled for TBR-2 and Sox-2 immunoreactivity. In C57 embryonic cortex, dense EdU-labeled cells were observed around the ventricular zone (VZ), with subventricular zone (SVZ) displaying moderate EdU-expression. Both the LGE and MGE expressed dense EdU-labeled cells. In autistic BTBR (E16 and E18), the VZ was thinner (~50%) and expressed fewer/diffuse EdU-labeling in VZ, SVZ and IMZ. The size of LGE and MGE were also smaller in BTBR and had significantly fewer EdU-labeled cells. Interestingly, these EdU-IR cells did not display the mediolateral migratory pattern. In C57 (E16 and E18), the Sox-2- and TBR-2-immunoreactive (IR) cells were observed in VZ and SVZ, respectively. In autistic BTBR, the VZ was thinner (~50%) and had fewer Sox-2-IR radial glial cells compared to C57. However, these radial glial cells were observed dispersed from VZ to IMZ indicating high migratory pattern towards cortical layer. The TBR-2-IR SVZ layer was also thinner in BTBR and had fewer intermediate progenitor cells compared to C57.The abnormal development of E16/E18 embryonic cortex seen in BTBR (1. decreased rate of neurogenesis in VZ, LGE, MGE; 2. decreased size of LGE and MGE; 3. decreased number of intermediate progenitor cells derived from radial glial cells; 4. increased rate of migration of radial glial cells

from VZ to IMZ) may be contributing factors that may predispose the BTBR with expression of ASD-like behaviors.

Disclosures: J. Lee: None. **S. Cheng:** None.

Poster

P047. Autism: Physiology and Systems III

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P047.02

Topic: A.07. Developmental Disorders

Support: IITKGP/SRIC/SGIGC-2015/DMN
IA/I/11/2500270
MHRD/JRF-SRF
DBT/JRF-SRF

Title: Alterations in connectivity in the deeper layers of the developing in motor cortex in an ASD model of mouse

Authors: H. K. SRIVASTAVA[1], Y. SELVARAJAN[2], *S. BANDYOPADHYAY[2];
[1]Advanced Technol. Develop. Ctr., Indian Inst. of Technol. Kharagpur, Kharagpur, India; [2]IIT Kharagpur, Kharagpur, India

Abstract: Autism spectrum disorders (ASD) is a set of neurodevelopmental disorders are characterized by atypical repetitive behavior and motor movements along with impairments in speech and reciprocal communication skills as its core symptoms. Meaningful motor movements require correlated and sequential neural activity patterns in the motor cortex. Recurrent neural connections are critical in generation of correlated and sequential activity patterns in a network. Developmental aberrations in these recurrent connections within the motor cortex leading to an altered network structure could underlie atypical motor movements observed in ASD. We investigated the intra-laminar and extra-laminar excitatory connections of the deeper layers of the mouse motor cortex using simultaneous 2-photon MNI-caged glutamate uncaging and Ca^{2+} imaging based circuit mapping, *in vitro*. Typically developing (P1-P10) motor cortex circuitry was compared with that of *in utero* Valproic Acid (VPA) exposed ASD model mice. We found that the targeted (uncaged) neurons responded with similar reliability across trials to MNI-glutamate stimulation suggesting an intact neuronal firing thresholds but the response strengths of the connected neurons were significantly different (lower) in the VPA treated P1-P5 group as compared to control group. However, the probability of connections between neurons was significantly higher in the VPA treated P1-P5 age group as compared to control. Further, in the control groups, the connection probability increased with age whereas in the VPA treated groups, the connection probability decreased with age indicating an altered circuit development and

network architecture in the motor cortex in ASD. Such alterations within motor cortex circuitry in ASD could form the basis of atypical motor behaviour and also altered vocalization sequence generation in the pup isolation calls of the mouse model of ASD.

Disclosures: H.K. Srivastava: None. **Y. Selvarajan:** None. **S. Bandyopadhyay:** None.

Poster

P047. Autism: Physiology and Systems III

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P047.03

Topic: A.07. Developmental Disorders

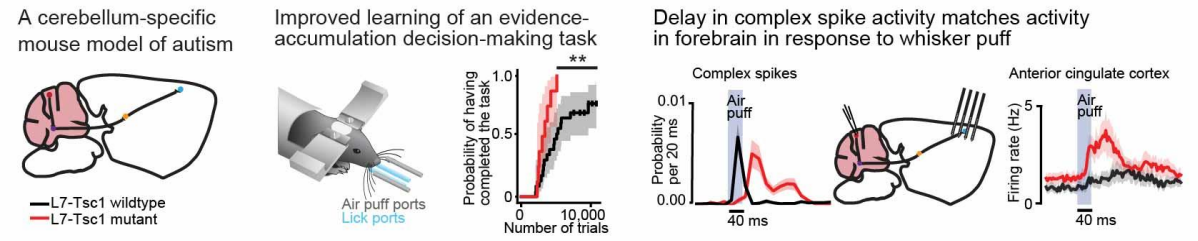
Support: NIH U19 NS104648
NIH R01 NS045193
NIH R01 MH115750
EU Horizon 2020 No. 844318

Title: Improved learning and increased sensory sensitivity in a cerebellar mouse model of autism

Authors: *M. OOSTLAND[1], M. KISLIN[1], Y. CHEN[1], T. CHEN[2], B. DEVERETT[3], S. S.-H. WANG[1];
[1]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ; [2]Dept. of Neurolog. Surgery, Univ. of California, San Fransisco, CA; [3]Dept. of Anesthesiol., Stanford Univ., Palo Alto, CA

Abstract: Autism is associated with an increased sensory sensitivity with occasional islands of enhanced function. Here we report that a cerebellum-specific mouse autism model with progressive Purkinje cell degeneration, L7-Tsc1, shows enhanced learning on a sensory evidence-accumulation task (Deverett et al., 2018, eLife; Deverett et al., 2019, Nat Comm; L7-Tsc1 wildtype mice: median number of trials to reach final level of the task: 5106, n=23 mice; L7-Tsc1 mutant mice: 3410 trials, n=8 mice; $p < 0.01$). L7-Tsc1 mutant mice also had more eye blinks in response to air puffs to their whiskers (L7-Tsc1 wildtype mice: median number of eye blinks in response to an air puff of 25 psi, 15 ms: 2, n=15 mice; L7-Tsc1 mutant mice: 9 eye blinks, n=7 mice; $p<0.01$), suggesting increased sensory sensitivity. Faster learning was emulated in wildtype mice by increasing stimulus intensity (C57BL/6J mice, air puffs at 10 psi (normal intensity): 4275 trials, n=9 mice; C57BL/6J mice, air puffs at 20 psi: 2225 trials, n=9 mice; $p < 0.01$) or by pairing stimuli with cerebellar optogenetic activation (*Pcp2-Cre x Ai27D* wildtype mice: 3311 trials, n=5 mice; *Pcp2-Cre x Ai27D* transgenic mice: 2512 trials, n=6 mice; $p < 0.01$). Enhanced learning was accompanied by delays in complex-spike firing in Purkinje cells (n=12 cells from 5 L7-Tsc1 wildtype mice and n=8 cells from 4 L7-Tsc1 mutant mice; as well as n=13 cells from 4 *Pcp2-Cre x Ai27D* transgenic mice) whose time course was similar to that of increased activity in the anterior cingulate cortex (n=262 cells from 5 L7-Tsc1 wildtype

mice and n=312 cells from 6 L7-Tsc1 mutant mice; as well as n=260 cells from 4 *Pcp2-Cre x Ai27D* transgenic mice), a prefrontal region known to express evidence- and choice-related activity. Two cerebellum-specific forms of learning, delay eyeblink conditioning and delay tactile startle conditioning, were still impaired in L7-Tsc1 mutant mice compared with wildtype littermates. These results show that a brain-region-specific alteration that leads to cognitive and social endophenotypes can also selectively improve specific domains of function.



Disclosures: M. Oostland: None. **M. Kislin:** None. **Y. Chen:** None. **T. Chen:** None. **B. Deverett:** None. **S.S. Wang:** None.

Poster

P047. Autism: Physiology and Systems III

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P047.04

Topic: A.07. Developmental Disorders

Support: Simons Foundation Autism Research Initiative-Research Award

Title: Developmental progression of prefrontal circuit dysfunction in a SHANK3$^{-/-}$ mouse model of Autism Spectrum Disorder

Authors: *G. DEVIENNE, J. R. HUGUENARD;
Wu Tsai Neurosciences Inst., Stanford Univ., Palo Alto, CA

Abstract: Autism Spectrum Disorders (ASDs) are phenotypically and genetically heterogeneous behavioral disorders of neural development. A leading candidate gene is Shank3, with mutations occurring in 1 - 2% of affected individuals. Shank genes encode scaffolding proteins located at the postsynaptic density of glutamatergic synapses. Shank3 complete knockout mice (Shank3$^{-/-}$) exhibit phenotypes of neurodevelopmental disorders such as ASD. Despite numerous studies focusing on ASD Shank3-/- related pathophysiological circuit mechanisms in mature mice, little is known regarding ASD onset and its evolution through the development. Our team aims to fill this knowledge gap by studying the effect of Shank3$^{-/-}$ genetic mutation at different stages of postnatal development in a murine Shank3 model. We performed our study in the medial prefrontal cortex (mPFC), which plays an important role in social and cognitive behavior and

displays a critical period of development that coincides with the onset of ASD behaviors. We used local field potential (LFP) analysis in acute brain slices of adult wild type (WT) and Shank3$^{-/-}$ mice. We used linear silicon arrays, which allowed us to characterize mPFC function across the entire cortical column. Intracortical stimulation resulted in a distinct LFP laminar profile, which was deconstructed via current source density (CSD) analysis to identify local sources and sinks of neuronal signals. Pharmacological dissection isolated distinct presynaptic (fiber volley) and postsynaptic (excitatory and inhibitory) sources/sinks, each with a specific spatiotemporal profile. We identified a global mPFC hyperfunction, affecting pre- and post-synaptic (by ~1.5x at maximum stimulus) components, with greatest deficits seen in deeper neocortical layers (by ~2x). Such hyperexcitability is coherent with other studies and might explain a comorbid epileptic disorder. We began to investigate early time points; a first screening at P10 showed no significant difference suggesting that mPFC circuit dysfunctions appear later. P14 time point was next tested, as the Shank3 isoforms reach their maximal expression at this age. Interestingly, our preliminary data show a transient hypofunction of the mPFC at P14, suggesting that mPFC dysfunction may result from reduced early circuit activity that could disrupt normal circuit formation contributing to the acquisition of ASD features in adults. This project will reveal how postnatal circuit alteration is involved in ASD and, through ultimate identification of specific circuit elements/synapses responsible for early developmental changes, may help to identify possible therapeutic targets or windows.

Disclosures: G. Devienne: None. **J.R. Huguenard:** None.

Poster

P047. Autism: Physiology and Systems III

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P047.05

Topic: A.07. Developmental Disorders

Support: Brain and Behavior Research Foundation's NARSAD Young Investigator Award
Hartwell Foundation's Individual Biomedical Award
University of Wisconsin System's WiSys Technology Foundation
Carla and Mike Austin Faculty Fellowship
Eunice Kennedy Shriver National Institute of Child Health and Human Development

Title: Brainstem white matter microstructure predicts sensory features in autistic adolescents

Authors: *O. SURGENT[1], D. DEAN, III[2], K. AUSDERAU[3], A. ALEXANDER[4], B. TRAVERS[3];
[1]Neurosci. Training Program, [2]Dept. of Pediatrics, [3]Occup. Therapy Program in the Dept. of Kinesiology, [4]Univ. of Wisconsin-Madison, Madison, WI

Abstract: Autistic youth often demonstrate differences in sensory features that impact adaptive behaviors and quality of life (Baranek et al., 2006; Ismael et al., 2018). Recent work has demonstrated associations between sensory features and brainstem white matter in both autistic children and autistic infants (Surgent et al., in prep; Wolff et al., 2017). Specifically, more prominent sensory features have been associated with higher white matter structural coherence (higher fractional anisotropy [FA], lower mean diffusivity [MD]) in the middle cerebellar peduncle (MCP) and pontine white crossing tract (PCT). However, it is unclear whether these relationships remain throughout adolescence, a time of white matter maturation and development. Therefore, the present study investigated the relationship between brainstem white matter and sensory features in autistic adolescents. We collected information about sensory features via the Sensory Experience Questionnaire 3.0 and diffusion weighted imaging brain scans from a pilot sample of 26 autistic adolescents (ages 13-17 years). In addition to analyzing the relationship between sensory features and MCP and PCT using classical diffusion tensor imaging metrics (FA and MD), we implemented multi-compartmental modeling to extract neurite-specific measures of orientation dispersion index (ODI) and intracellular volume fraction (ICVF). The PCT was the only white matter area to show relationships with sensory features. Sensory features were negatively associated with MD (r=-.49, p=.01; Fig A) and positively associated with both ODI (r=.47, p=.02; Fig B) and ICVF (r=.48, p=.01; Fig C). Results indicate that the relationship between sensory features and brainstem white matter in autistic youth remains consistent throughout adolescence. Further, results suggest that increased white matter coherence in addition to increased neurite density and dispersion in the brainstem underlies sensory features. Future analyses will seek to further evaluate these relationships in a larger sample of autistic adolescents.

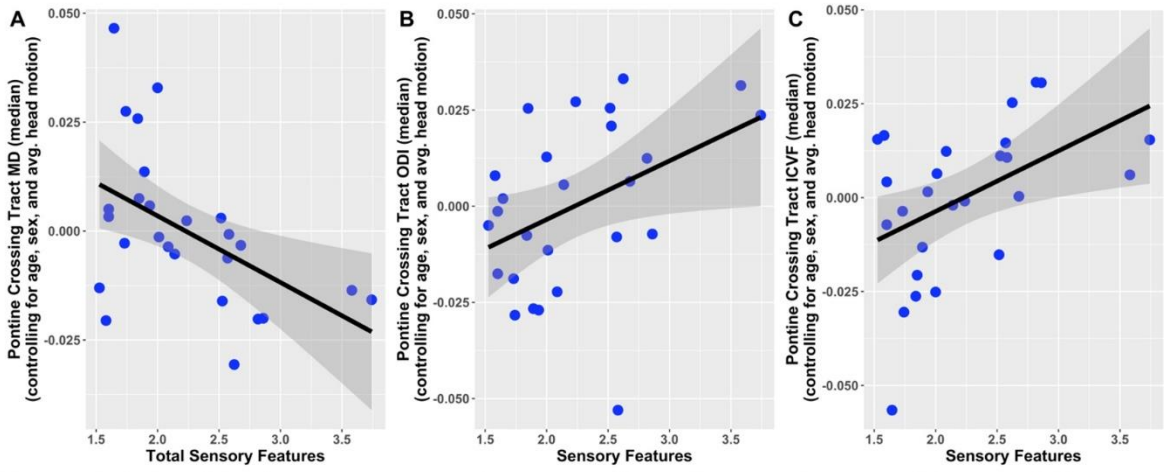


Figure. Relationships between sensory features and pontine crossing tract (A) mean diffusivity, (B) neurite orientation dispersion index, and (C) intracellular volume fraction in autistic adolescents.

Disclosures: O. Surgent: None. **D. Dean:** None. **K. Ausderau:** None. **A. Alexander:** None. **B. Travers:** None.

Poster

P047. Autism: Physiology and Systems III

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P047.06

Topic: A.07. Developmental Disorders

Support: U01DA 041022
U01DA041028
U01DA041048
U01DA041089
U01DA041106
U01DA041117
U01DA041120

Title: Atypical emotional modulation of cognitive control in children with anxiety and high autistic traits

Authors: *T. S. MULLINS, J. HOGEVEEN;
Univ. of New Mexico, Albuquerque, NM

Abstract: Autism and anxiety independently affect a large number of children and children with autism are at increased risk for anxiety disorders. Yet, the degree to which anxiety's underlying neural mechanisms and clinical symptoms are transdiagnostic, or distinct in youths with comorbid autism, remains unclear. Here, we determined whether a classic finding in the cognitive neuroscience of anxiety—increased salience and attention to negative emotional stimuli—varies across children who do or do not also have elevated autistic traits. Specifically, we used the *Adolescent Brain Cognitive Development* (ABCD) study dataset to compare threat or negative emotion bias across 4 well-matched subgroups of 9-10 year old children: 1) high anxiety and low autistic traits (ANX; *N*=54), 2) high autistic traits and low anxiety (AUT; *N*=54), 3) high anxiety *and* autistic traits (ANX+AUT; *N*=54), and 4) low anxiety and low autistic traits (CTRL; *N*=54). This enabled us to isolate the effects of anxiety, autistic traits, and their interaction on the behavioral and neural correlates of emotional modulation of cognitive control using the ABCD study's emotional *n*-back (EN-Back) fMRI paradigm. Behavioral data indicated that fearful faces enhanced cognitive control performance specifically within the ANX group, but not in the ANX+AUT group, suggesting that the attention to threat bias often found in the anxiety literature may not be a characteristic of comorbid autism and anxiety. Neuroimaging analyses are ongoing and will hypothesize differential recruitment of prefrontal-amygdala circuitry in response to negative emotional faces in ANX relative to CTRL groups, and a lack of differences in these circuits between ANX+AUT and AUT. Our preliminary findings indicate that the attention to threat bias may be unique to children with anxiety and low levels of autistic traits, and may not be a mechanism driving anxiety in children with comorbid autism. This

would indicate a need for more transdiagnostic mechanistic studies to develop novel precision medicine approaches for treating children with anxiety and comorbid autistic traits.

Disclosures: T.S. Mullins: None. **J. Hogeveen:** None.

Poster

P047. Autism: Physiology and Systems III

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P047.07

Topic: A.07. Developmental Disorders

Support: P50MH100023
P50MH100023
UL1TR002378
ProMedica Gift

Title: Dose-dependent effects of oxytocin on brain function during an interactive social game in Autism Spectrum Disorder

Authors: *E. ANDARI[1], A. S. COTTON[2], J. F. CUBELLS, JR[3], J. K. RILLING[4], L. J. YOUNG[5];
[1]Univ. of Toledo, Ruppert Hlth. Ctr., Toledo, OH; [2]Univ. of Toledo, Toledo, OH; [3]Dept. of Psychiatry and Beh. Sci., Emory Univ. Sch. Med., Dept. of Human Genetics, Emory Univ. Sch. Med., Atlanta, GA; [4]Dept. of Anthropology, Emory Univ., Atlanta, GA; [5]Ctr. for Translational Social Neurosci., Emory Univ., Atlanta, GA

Abstract: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder that is characterized by deficits in social interaction and communication. Oxytocin is a key modulator of social behavior. Acute administration of intranasal oxytocin (IN-OT) enhances social functioning in autism. However, long-term trials with IN-OT have yielded some inconsistent findings. Here, we conducted a dose-dependent study with IN-OT to examine target engagement for this treatment on the brain activity of adults with ASD. A total of 31 ASD participants were recruited from the Emory Autism Center in Atlanta. ASD subjects underwent four fMRI scanning sessions in which they completed an interactive social ball game. The social ball game consisted of playing with groups of individuals who gave either positive or negative feedback during the game. Participants also played the same game with computers. All patients received 4 doses of IN-OT (8IU, 24IU and 48IU) and IN-placebo in a randomized, double-blind, within-subject, placebo-controlled design. Visits were separated by a one-week interval. Participants played the ball game inside the MRI scanner (3T, Siemens) 60 minutes after receiving IN spray.ROI analysis on the basolateral amygdala, ventral anterior insula and ACC yielded a significant dose-dependent effect for oxytocin in response to social feedback, as compared to

computer feedback. Specifically, higher doses of IN-OT (48IU) enhanced the activity of these areas in response to negative and positive social feedback, compared to placebo and smaller doses. These findings show promising effects of IN-OT on brain function in Autism Spectrum Disorder in key brain areas known to be involved in social and emotional processes.

Disclosures: E. Andari: None. **A.S. Cotton:** None. **J.F. Cubells, Jr:** None. **J.K. Rilling:** None. **L.J. Young:** None.

Poster

P047. Autism: Physiology and Systems III

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P047.08

Topic: A.07. Developmental Disorders

Support: This work was supported by a grant from the Simons Foundation (SFARI 614379), awarded to Joshua Hartshorne and Stefano Anzellotti.

Title: Contrastive AI reveals the structure of individual variation in Autism Spectrum Disorder

Authors: *A. AGLINSKAS, J. HARTSHORNE, S. ANZELLOTTI;
Boston Col., Boston, MA

Abstract: Autism Spectrum Disorder is highly heterogeneous. Individuals with ASD differ in the severity of social and behavioral symptoms, and in their cognitive abilities. Most of the work done in this area focuses on comparisons between ASD and typically developing (TD) controls. Individual variation within ASD is less studied, but it is likely key to inform diagnosis and personalized interventions. However, ASD-specific neuroanatomical variation is entangled with variation due to other causes (e.g. age and gender), making it challenging to tell apart anatomical dimensions that are due to ASD, from those that are shared with the TD population. In this work we trained a novel deep neural network architecture to disentangle ASD-specific neuroanatomical variation from variability shared with typically developing (TD) participants. We trained a contrastive variational autoencoder (CVAE) on the ABIDE dataset (N=539 TD and N=470 ASD subjects) using brains extracted from anatomical T1w scans. For each subject, the CVAE separately learned a set of anatomical features that are common to both TD and ASD participants (like age and gender), and a set that are unique to ASD. Representational similarity analyses revealed that ASD-specific neuroanatomical features identified in this way capture individual differences in autism severity (ADOS) and symptoms (DSM-IV behavioral subtypes) irrespective of confounding variability in age, gender and scanner-type (these confounding variables were better captured by the CVAE's shared features).Next, to identify interpretable anatomical loci of individual variation - we reconstructed the anatomy of ASD participants only using shared features, generating “synthetic twin” brains without autism. Thanks to this

procedure, we identified two continuous dimensions of variation among ASD participants: one associated with structural changes in the thalamus; and second affecting the right somatosensory cortex and right superior longitudinal fasciculus.

Disclosures: A. Aglinskas: None. **J. Hartshorne:** None. **S. Anzellotti:** None.

Poster

P047. Autism: Physiology and Systems III

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P047.09

Topic: A.07. Developmental Disorders

Support: National Science Foundation Graduate Research Fellowship to FdU
Ford Foundation Predoctoral Fellowship to FdU
NIH R01 NS045193 to SS-HW
NIH R01 MH115750 SS-HW
Fellowship by New Jersey Autism Center of Excellence (CAUT20AFP006) to H-JB
Netherlands Organization for Scientific Research—Veni ZonMW, 91618112
Erasmus MC Fellowship 106958 to H-JB

Title: Specific Loss of Thalamic Modulation of Cerebellum-Neocortex Codevelopment in Autism Spectrum Disorder

Authors: *B. LI[1], F. D'OLEIRE UQUILLAS[1], E. BUEICHEKÚ[2], J. SEIDLITZ[3], H.-J. BOELE[1], J. VERPEUT[4], M. LATIF[1], R. GESUE[1], K. STEELE[1], M. TROTTER[1], T. FASULO[1], J. YAO[1], J. SEPULCRE[2], S. S.-H. WANG[1], J. GOMEZ[1];
[1]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ; [2]Dept. of Radiology, Gordon Ctr. for Med. Imaging, Massachusetts Gen. Hospital, Harvard Med. Sch., Boston, MA; [3]Children's Hosp. of Philadelphia, Univ. of Pennsylvania, Philadelphia, PA; [4]Dept. of Psychology, Arizona State Univ., Tempe, AZ

Abstract: A diaschisis in cerebellar-neocortical pathways, related to Autism Spectrum Disorder (ASD), may be modulated by thalamic structural integrity. Previous tract-tracing work in mice has found that cerebellar-fugal pathways traverse specific thalamic nuclei. These pathways emerge from the coordinated development of multiple brain regions subserving a common functional specialization. Using structural MRI, we investigate the relationship between morphometric covariance in typically developing (TD) and ASD children amongst specific cerebellar lobules, thalamic nuclei, and somatosensory cortex.
Methods: T1w MRI data from 69 children from ABIDE (94% males, 40% controls, median age=8±2;range:5-17 years) were used to compute volume density (mm3) at every brain region of

the HCP-180, SUIT-Cerebellar-13, and Iglesias-Thalamic-25 atlases. Separately in TD and ASD, we assessed the following lateralized pathways based on covariation of brain volumes: Cerebellar Lobule I-IV, Crus I, and Lobule VI structural volume values were modeled as independent variables in predicting somatosensory cortex (SSC) volume, with thalamic nuclei volumes modeled as moderators: ventral-posterolateral (VPL), ventromedial (VM), medial-ventral reuniens (MvR), mediodorsal medial magnocellular (MDm), mediodorsal lateral parvocellular (MDl), laterodorsal (LD), medial geniculate (MGN), lateral geniculate (LGN), and anteroventral (AV). Results were corrected for multiple comparisons (FDRq < 0.05).
Results: In TD children, we found that the covariation between left/right cerebellar Lobules I-IV and contralateral SSC was moderated by contralateral thalamic MvR nuclei values ($p < 0.05$, Cohen's effects > 0.4). Covariation between left/right Crus I and contralateral SSC was moderated by contralateral MvR ($p < 0.05$, Cohen's effects > 0.4) and LGN values ($p < 0.05$, Cohen's effects > 0.5). We found similar relationships between Lobule VI and SSC with thalamic MDm and MGN ($p < 0.05$, Cohen's effects > 0.5). These relationships were not evident in ASD children ($p > 0.05$). Further, right SSC volume in ASD was significantly greater relative to TD ($p < 0.05$, cohen's effects = 0.48).
Conclusions: Consistent with the rodent literature, we show that the somatosensory cortex is a significant downstream target of the cerebellar-cortical pathway, and reveal for the first time that thalamic nuclei moderate volumetric trajectories between these distal brain regions. Disruptions in these pathways may result in social and cognitive impairment during development, such as in ASD. The ASD connectome across childhood may be leveraged as a biomarker of change in intervention studies.

Disclosures: B. Li: None. **F. d'Oleire Uquillas:** None. **E. Bueichekú:** None. **J. Seidlitz:** None. **H. Boele:** None. **J. Verpeut:** None. **M. Latif:** None. **R. Gesue:** None. **K. Steele:** None. **M. Trotter:** None. **T. Fasulo:** None. **J. Yao:** None. **J. Sepulcre:** None. **S.S. Wang:** None. **J. Gomez:** None.

Poster

P146. Epilepsy and Seizures: In Vivo

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P146.01

Topic: B.10. Epilepsy

Title: Neurophysiological and neurobiochemical alterations in cerebellar granule cells in kindled mice

Authors: *M. AGUERO MEDINA[1], J. ACOSTA MAGALLANES[1], C. RUBIO OSORNIO[1], L. HERNÁNDEZ LÓPEZ[1], J. BRITTO BRITTO[1], N. RIVERA SOTELO[2], V. CUSTODIO RAMÍREZ[1];

[1]Neurofisiología, [2]Neurocirugía, Inst. Nacional de Neurología y Neurocirugía MVS, Ciudad de México, Mexico

Abstract: The cerebellum plays a major role in the control of epilepsy. Epileptic patients have shown activation of the neocortical-pontocerebellar pathway, evidencing extensive participation of the cerebellum and the middle cerebellar peduncle in the neuropathophysiology of epilepsy. Afferent fibers of the cerebellum pass through the mossy fibers, exciting the granule and Golgi cells, both cell types are part of the cerebellar cortex. The aim of this study was to identify and analyze the neurophysiology and neurobiochemical changes produced after the kindling model in granule cells of the cerebellum in mice. In order to test this, we carried out calcium imaging and immunohistochemistry. The kindling model was carried out placing bipolar electrodes in the left basal lateral amygdala. Subsequently, the mice received a daily electric stimulus until each mice presented generalized tonic-clonic seizures. Two groups of mice cerebellum slices were used for calcium imaging. The cerebellum slices were incubated in (Flou 4-AM) a fluorescent calcium indicator. Thus, active cells were quantified by increased fluorescence. The second group of control and kindled mice were sacrificed by intracardiac perfusion to carry out an immunohistochemistry essay to quantify the expression of glutamate, GABA, c-Fos, GFAP, nNOS, iNOS, and the GABAA receptor in the cerebellum and cortex. All procedures followed the International Guide for the Care and Use of Laboratory Animals (NIH publication No. 86-23, revised 1987). Our results showed increased activity in cerebellar granule cells in kindled mice compared to the control group. Additionally, we also found significant increases in the immunopositivity expression of glutamate, GFAP, and GABAA receptor in granular cells of the cerebellum in addition an increasing trend was found of nNOS and iNOS. Moreover, a significant decrease in GABA and c-Fos was observed. In the cerebral cortex, we found a significant increase in the expression of GABA, c-fos, GFAP, and GABA receptors and also a positive trend of increased nNOS and iNOS, while glutamate concentration did not present significant changes. The cerebellar granular hyper-synchronization is explained by the increase in neuronal coactivity as a result of the imbalance of neurotransmitters for excitation and inhibition of granule cells. These results suggest that changes in the frequency of potential actions and the coactivity in the granule cells of kindled mice, lead to neurophysiological and neurochemical changes.

Disclosures: M. Aguero Medina: None. **J. Acosta Magallanes:** None. **C. Rubio Osornio:** None. **L. Hernández López:** None. **J. Britto Britto:** None. **N. Rivera Sotelo:** None. **V. Custodio Ramírez:** None.

Poster

P146. Epilepsy and Seizures: In Vivo

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P146.02

Topic: B.10. Epilepsy

Support: NS050229

Title: Dysregulation of tau expression and S202/T205 phosphorylation levels in the hippocampus of a rat model of temporal lobe epilepsy

Authors: F. A. CONCEPCION, M. N. KHAN, N. A. EKSTROM, *N. P. POOLOS; Neurol., Univ. of Washington, Seattle, WA

Abstract: Studies have shown altered tau expression and phosphorylation in human epilepsy. We investigated in a pilocarpine status epilepticus (SE) rat model of acquired epilepsy the changes in total tau expression and tau phosphorylation at the S202/T205 human locus, known to be hyperphosphorylated in Alzheimer's disease. We used western blotting to analyze tau expression within the hippocampus at two time points of chronic epilepsy: 6-9 weeks post-SE and 14-17 weeks post-SE. We also used mass spectrometry (MS) to identify tau phosphosites in both rat model and brain tissue from human epilepsy patients to determine the correspondence between the two species. In the whole hippocampus at 6-9 weeks post-SE, we observed reduced total tau levels (82.1 ± 3.2% of naïve control; n=8; p=0.0008) and S202/T205 phosphorylated tau (p-tau) levels (72.2 ± 11.2% of controls, n=8; p=0.043). In 14-17 weeks post-SE, we noticed a loss of p-tau levels (67.9 ± 13.2% of control, n=4; p=0.094) that was not significant, but no changes in total tau amounts (93.3 ± 8.0% of control, n=4; p=0.47). Investigating hippocampal subregions of 6-9 week post-SE rats, we observed a loss in total tau expression in CA1 (79.1 ± 5.2% of naïve levels; n=4; p=0.027), and although nonsignificant, reduced p-tau levels (65.7 ± 14.3% of naïve levels; n=4; p=0.096). In CA3 there was a greater loss of p-tau (57.7 ± 14.8% of naïve; n=5; p=0.046), and a trend towards a drop in total tau (80.2 ± 10.7% of controls; n=5; p=0.14). With MS, we identified 29 rat tau phosphosites (p-sites) and 23 human tau p-sites (n=2 samples for both rat and human). Of the 26 rat p-sites that had corresponding potential p-sites in humans, 17 (65%) showed phosphorylation in their human counterparts. Conversely, of the 21 human p-sites that had corresponding potential p-sites in rats, we observed phosphorylation in their rat counterparts in 17 (81%) of these human p-sites. We conclude that tau expression is dysregulated in our rat epilepsy model, with differing changes in expression in hippocampal subregions. Tau dysregulation persists at two time points of chronic epilepsy. Identification of 29 rat tau phosphosites and 23 human tau phosphosites indicates a high degree of correspondence in tau phosphosite patterns between rats and humans. These data indicate that a rat model of acquired epilepsy will enable future correlation of changes in tau expression and phosphorylation with the development of epilepsy after a brain insult.

Disclosures: F.A. Concepcion: None. **M.N. Khan:** None. **N.A. Ekstrom:** None. **N.P. Poolos:** None.

Poster

P146. Epilepsy and Seizures: In Vivo

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P146.03

Topic: B.10. Epilepsy

Title: Loss of hippocampal efferent projections in the mouse intrahippocampal kainic acid model

Authors: *A. M. FERNANDEZ[1], C.-A. N. GUTEKUNST[2], D. P. GROGAN[3], N. P. PEDERSEN[4], R. E. GROSS[5];
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Abstract: Unilateral intrahippocampal injection of kainic acid is used as a model of medial temporal lobe epilepsy and provides a platform to study the mechanisms of epilepsy. Here, we used an AAV-9 EYFP-tagged viral vector as an anterograde tracer to map out the efferent hippocampal projections after the development of spontaneous seizures in this model in C57BL/6J male mice. The purpose of the study was to identify the extent of changes in hippocampal efferent system in several brain regions that receive significant inputs from the hippocampus. Loss of efferent hippocampal fibers was greatest in the retrosplenial cortex where neuronal loss was also observed. Loss of fibers was also observed in the fornix without any specific effect in the lateral mammillary nuclei. Although expected, these observations provide further evidence of the broader network effects as a result of hippocampal cell loss.

Disclosures: A.M. Fernandez: None. **C.N. Gutekunst:** None. **D.P. Grogan:** None. **N.P. Pedersen:** None. **R.E. Gross:** None.

Poster

P146. Epilepsy and Seizures: In Vivo

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Program #/Poster #: P146.04

Topic: B.10. Epilepsy

Support: NIH Grant R15 GM110651
NIH Grant R01 NS072129

Title: O-glcnac transferase ogt-1 and the ubiquitin ligase eel-1 modulate seizure susceptibility in c. elegans

Authors: *N. SUTHAKARAN[1], J. WIGGINS[1], A. GILES[2], K. OPPERMAN[3], B. GRILL[4], K. DAWSON-SCULLY[1];

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Abstract: Neurodevelopmental disorders such as epilepsy and autism have been linked to an imbalance of excitation and inhibition (E/I) in the central nervous system. The simplicity and tractability of *C. elegans* allows our electroconvulsive seizure (ES) assay to be used as a behavioral readout of the locomotor circuit and neuronal function. *C. elegans* possess conserved nervous system features such as gamma-aminobutyric acid (GABA) and GABA receptors in inhibitory neurotransmission, and acetylcholine (Ach) and acetylcholine receptors in excitatory neurotransmission. Our previously published data has shown that decreasing inhibition in the motor circuit, via GABAergic manipulation, will extend the time of locomotor recovery following electroshock. Similarly, mutations in a HECT E3 ubiquitin ligase called EEL-1 leads to impaired GABAergic transmission, E/I imbalance and altered sensitivity to electroshock. Mutations in the human ortholog of EEL-1, called HUWE1, are associated with both syndromic and non-syndromic intellectual disability. Both EEL-1 and its previously established binding protein, OGT-1, are expressed in GABAergic motor neurons, localize to GABAergic presynaptic terminals, and function in parallel to regulate GABA neuron function. In this study, we tested behavioral responses to electroshock in wildtype, *ogt-1*, *eel-1* and *ogt-1; eel-1* double mutants. Both *ogt-1* and *eel-1* null mutants have decreased inhibitory GABAergic neuron function and increased electroshock sensitivity. Consistent with EEL-1 and OGT-1 functioning in parallel pathways, *ogt-1; eel-1* double mutants showed enhanced electroshock susceptibility. Expression of OGT-1 in the *C. elegans* nervous system rescued enhanced electroshock defects in *ogt-1; eel-1* double mutants. Our *C. elegans* electroconvulsive seizure assay was the first to model a human X-linked Intellectual Disability (XLID) associated with epilepsy and suggests a potential novel role for the OGT-1/EEL-1 complex in seizure susceptibility.

Disclosures: N. Suthakaran: None. **J. Wiggins:** None. **A. Giles:** None. **K. Opperman:** None. **B. Grill:** None. **K. Dawson-Scully:** None.

Poster

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Program #/Poster #: P146.05

Topic: B.10. Epilepsy

Support: NIH Grant R01NS099586

Title: Respiratory alkalosis as a tool to interrogate absence seizure-generating neural circuits

Authors: K. A. SALVATI[1], R. J. SKYBERG[2], I. P. LESMANA[1], ***M. BEENHAKKER**[1]; [1]Univ. of Virginia Sch. of Med., Charlottesvle, VA; [2]Uva, Charlottesville, VA

Abstract: Hyperventilation reliably provokes seizures in patients with absence epilepsy. To understand the physiological parameters that drive hyperventilation-provoked absence seizures we developed rat and mouse models that recapitulate the predictable human seizure response. We now use these models to both (1) resolve the cellular mechanisms that contribute to hyperventilation-provoked seizures, and (2) resolve neural circuits that initiate and propagate absence seizures. In seizure-prone rats (WAG/Rij) and mice (*Gria4* mutant), absence seizures are primarily sensitive to the respiratory alkalosis that accompanies hyperventilation. In both rodent models, hypoxia-induced hyperventilation provokes a burst of spike-wave discharges (SWDs), electrographic correlates of absence seizures, that is also associated with arterial alkalization. Consistent with the hypothesis that SWDs are specifically provoked by alkalosis, supplementing hypoxic conditions with high CO_2 suppresses the SWD response; high CO_2 alone is also sufficient to reduce SWDs in the rodents. Activity-dependent labeling approaches (i.e., cFos) suggest that hyperventilation activates neurons in the intralaminar thalamic nuclei to provoke SWD-generating neural circuits. Using multielectrode silicone recording probes in head-fixed *Gria4* mutants, we are leveraging the predictability of hyperventilation-provoked SWDs to identify neural circuit activity patterns that precipitate seizures. Our recordings show that spontaneous (i.e., during normal breathing conditions) and hyperventilation-provoked SWDs follow a stereotyped temporal progression of activity. Also, preliminary recordings indicate that the midline thalamic nuclei, including some intralaminar nuclei, produce SWDs before the laterally-situated sensory nuclei. Collectively, our data support a model wherein pH sensitive neurons of midline thalamic structures precipitate hyperventilation-provoked absence seizures.

Disclosures: K.A. Salvati: None. **R.J. Skyberg:** None. **I.P. Lesmana:** None. **M. Beenhakker:** None.

Poster

P146. Epilepsy and Seizures: In Vivo

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Topic: B.10. Epilepsy

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CURE Taking Flight Award
AES Junior Investigator Award

Title: Entorhinal-hippocampal desynchronization in chronically epileptic mice

Authors: *S. Y. FENG, L. PAGE-HARLEY, K. DIEGO, S. LAMSIFER, T. SHUMAN; Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Temporal lobe epilepsy is one of the most common types of epilepsy in adults and causes pervasive memory impairments that significantly impact the quality of life. Pilocarpine-treated epileptic mice show altered hippocampal synchronization with reduced theta coherence and abnormal interneuron firing between the CA1 and dentate gyrus (DG) region of the hippocampus (HPC). However, it remains unclear whether synchronization deficits in HPC reflect impaired inputs from the entorhinal cortex and when these deficits emerge following epileptogenesis. The medial entorhinal cortex (MEC) is the upstream region sending direct spatial inputs into HPC, which shows severe cell death and cell hyperexcitability in epilepsy. Cognitive processes require precise communication between circuits, suggesting that altered timing between HPC and MEC may contribute to epilepsy-associated cognitive deficits. We have performed acute in vivo electrophysiology with silicon probes to simultaneously record from 512 channels in HPC and MEC of epileptic and control mice running in virtual reality. Wireless EEG devices were implanted in all animals to monitor chronic seizure activity. Two time points (3 weeks and 8 weeks post pilocarpine) were chosen to capture the early and late stages of epilepsy. Preliminary analysis using local field potentials (LFPs), multiunit activity, and coherence measures revealed that epileptic mice had altered LFP power and synchronization between the MEC and HPC. In the hippocampus, theta power deficits were progressive, with significant changes found only at the 8-week time point. Gamma power, on the other hand, was reduced in CA1 and DG by 3 weeks post-pilocarpine, likely reflecting interneuron death early after pilocarpine. At 8 weeks post pilocarpine, we also found reduced theta phase coherence between MEC layers II/III (MECII, MECIII) and CA1, and reduced phase locking of MECII spiking to CA1 theta oscillations. Together, these data indicate a significant impairment in the timing of MEC inputs into HPC, which may contribute to the reduced coherence and altered spike timing we have previously found in epileptic mice. Future analysis will focus on single-unit analysis to determine whether excitatory and inhibitory neurons are specifically altered, and whether spatial coding in MEC is disrupted in epileptic mice.

Disclosures: S.Y. Feng: None. **L. Page-Harley:** None. **K. Diego:** None. **S. Lamsifer:** None. **T. Shuman:** None.

Poster

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Topic: B.10. Epilepsy

Support: R01 NS083402

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R03 NS103029
R21 NS104293
R21 NS109894

Title: Spontaneous seizure and memory loss in mice expressing an epileptic encephalopathy variant in the calmodulin-binding domain of Kv7.2

Authors: *E. KIM, H. CHUNG;
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Abstract: Epileptic encephalopathy (EE) is characterized by seizures that respond poorly to antiseizure drugs, psychomotor delay, and cognitive and behavioral impairments. One of the frequently mutated genes in EE is KCNQ2, which encodes the Kv7.2 subunit of voltage-gated Kv7 potassium channel. Kv7 channels composed of Kv7.2 and Kv7.3 are enriched at the axonal surface where they potently suppress neuronal excitability. Previously, we reported that de novo dominant EE mutation M546V in human Kv7.2 blocks calmodulin binding to Kv7.2 and axonal surface expression of Kv7 channels via their intracellular retention. However, whether these pathogenic mechanisms underlie epileptic seizures and behavioral comorbidities remains unknown. Here, we report conditional transgenic cKcnq2+/M547V mice, in which expression of mouse Kv7.2-M547V (equivalent to human Kv7.2-M546V) is induced in forebrain excitatory pyramidal neurons. These mice display early mortality, spontaneous seizures, enhanced seizure susceptibility, memory impairment, and repetitive behaviors. Furthermore, hippocampal pathology shows widespread intraneuronal aggregation of mutant Kv7.2-M547V proteins, neurodegeneration and reactive astrocytes. This study is the first to demonstrate that the impairment in axonal surface expression of Kv7 channels is associated with epileptic seizures, cognitive and behavioral deficits, and neuronal loss in KCNQ2-related EE.

Disclosures: E. Kim: None. **H. Chung:** None.

Poster

P146. Epilepsy and Seizures: In Vivo

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Title: Reduced hippocampal inhibition and enhanced autism-epilepsy comorbidity in mice lacking neuropilin 2

Authors: *C. EISENBERG[1], D. SUBRAMANIAN[3], M. AFRASIABI[4], J. DELUCIA[1], M. SHIFLETT[2], V. SANTHAKUMAR[3], T. TRAN[1];
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Abstract: The neuropilin receptors and their semaphorin ligands play key roles in brain circuit development by regulating numerous crucial neuronal processes, including synapse maturation and migration of GABAergic interneurons. Consistent with its developmental roles, the neuropilin 2 (Nrp2) locus contains polymorphisms in patients with autism spectrum disorder (ASD). Nrp2 deficient mice show autism-like behavioral deficits and propensity to develop seizures. In order to determine the pathophysiology in Nrp2 deficiency, we examined the hippocampal interneuron subtypes and inhibitory regulation of CA1 pyramidal neurons in mice lacking one or both copies of Nrp2. Immunostaining for interneurons revealed that Nrp2-/- mice have reduced number of parvalbumin, somatostatin and neuropeptide Y cells, mainly in CA1. Whole cell recordings identified reduced firing and hyperpolarized shift in resting membrane potential in CA1 pyramidal neurons from Nrp2+/- and Nrp2-/- mice compared to Nrp2+/+ indicating decrease in intrinsic excitability. Simultaneously, the frequency and amplitude of spontaneous inhibitory postsynaptic currents (sIPSCs) are reduced in Nrp2 deficient mice. A convulsive dose of kainic acid evoked electrographic and behavioral seizures with significantly shorter latency, longer duration and higher severity in Nrp2-/- compared to Nrp2+/+ animals. Finally, Nrp2+/- and Nrp2-/-, but not Nrp2+/+, mice have impaired cognitive flexibility demonstrated by reward-based reversal learning, a task associated with hippocampal function. Together these results demonstrate a broad reduction in interneuron subtypes and compromised inhibition in CA1 of Nrp2-/- mice, which could contribute to the heightened seizure susceptibility and behavioral deficits consistent with an ASD/epilepsy phenotype.

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Poster

P146. Epilepsy and Seizures: In Vivo

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Program #/Poster #: P146.09

Topic: B.09. Network interactions

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University of Strasbourg Institute for Advanced Study
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Title: Information processing dynamics in hippocampus and cortex and their alterations in experimental pathology

Authors: *W. CLAWSON, N. PEDRESCHI, T. MADEC, A. GHESTEM, P. QUILICHINI, D. BATTAGLIA, C. BERNARD;
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Abstract: Neurological disorders share common high-level alterations, such as cognitive deficits, anxiety, and depression. This raises the possibility of fundamental alterations in the way information conveyed by neural firing is maintained and dispatched in the diseased brain. Using experimental epilepsy as a model of neurological disorder we tested the hypothesis of altered information processing, analyzing how neurons in the hippocampus and the entorhinal cortex store and exchange information during slow and theta oscillations. We equate the storage and sharing of information to low level, or primitive, information processing at the algorithmic level, the theoretical intermediate level between structure and function. We find that these low-level processes are organized into substates during brain states marked by theta and slow oscillations. Their internal composition and organization through time are disrupted in epilepsy, loosing brain state-specificity, and shifting towards a regime of disorder in a brain region dependent manner. We propose that the alteration of information processing at an algorithmic level may be a mechanism behind the emergent and widespread co-morbidities associated with epilepsy, and perhaps other disorders.

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Poster

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Topic: B.09. Network interactions

Support: Marie Skłodowska-Curie grant agreement No713750

ANR- 11-IDEX-0001-02

Title: Dynamic multiplex network analysis of human intracranial recordings during postictal aphasia

Authors: *N. PEDRESCHI[1,2], A. TREBUCHON[1,3], A. BARRAT[2], D. BATTAGLIA[1,3];
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Abstract: Lesion related aphasia in humans has been widely investigated, while much remains unknown about the mechanisms that cause aphasia as a transient post-seizure symptom in epileptic patients. We analyse very long intracranial EEG recordings of a few patients suffering from pharmaco-resistant epilepsy and postictal aphasia, whose language capabilities are tested after a seizure until their full recovery. We study the functional connectivity between different cortical sites in a time and frequency resolved manner, to capture how the flexible dynamics of inter-regional interactions is affected by the seizure. Specifically, for each recording, we compute the wavelet coherence, in 5 frequency bands peaked around 1, 5, 10, 20 and 40 Hz, of each pair of electrodes at each time stamp, thus representing each recording as a time-varying, multilayer network (dynamic multiplex). Within each layer of each dynamic multiplex, we then compute the time series of various graph theoretical metrics, such as the instantaneous strength and modularity along with the instantaneous partition of the nodes into different modules. A wide increase in strength, and corresponding decrease in modularity, in all bands from seizure onset to offset is then followed by a phase of gradual recovery to baseline activity, whose duration increases with the frequency and can extend well beyond the disappearance of reported aphasia symptoms. We also characterized the flow between consecutive network frames, identifying and comparing different types of network dynamics. We studied in particular: the rate of overall reconfiguration of links from one frame to the next, or dynamic Functional Connectivity (dFC) speed; and the flexibility or crystallization of network modules through time, by means of a dynamic Allegiance matrix (dAm) analysis. A large variability, combined with a structured allegiance dynamics characterize the baseline "liquid" dynamics, while other dynamics types, more "gaseous" or "crystallized", appear during the seizure and recovery. Importantly, we recover a pathological state whose occurrence is correlated with the reported language-test fallacies. This state is characterized by a loss of allegiance (more evident in gamma and beta layers) between the temporal and parietal regions, which thus leave and enter different modules in an independent and not integrated manner. This state also corresponds to momentary drops of dFC-speed during the recovery phase. In conclusion, in our work we devise a data driven pipeline that can help both shed light on general mechanisms underlying postictal aphasia and address patient specific issues.

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Poster

P147. Epilepsy and Seizures: Animal Models

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Topic: B.10. Epilepsy

Support: Partially funded by VIEP-BUAP grant to CA en Neuroendocrinología BUAP-CA-288
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Title: Effect of prolactin on absence seizures in the taiep rat: an animal model of H-ABC leukodystrophy

Authors: *C. CORTES[1], E. S. HERNANDEZ[1], J. R. EGUIBAR[1,2];
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Abstract: The taiep rat, is a spontaneous myelin mutant from Sprague-Dawley strain obtained at the Benemérita Universidad Autónoma de Puebla, with an initial hypomyelination followed by a progressive demyelination in the central nervous system. This mutant is equal of human leukodystrophy hypomyelination with atrophy of the basal ganglia and cerebellum (ABC). We have already reported that taiep rats had spike-wave discharges, with a mean frequency of 6.25 Hz, like human absence seizures. Absence seizures are a generalized type of epilepsy characterized by sudden loss of consciousness and abnormal cortical electrical activity in both cerebral hemispheres with a mean frequency around 6 Hz. Absence seizures have been shown to be sexually dimorphic in humans, but the underlying mechanisms are not discerning until now. The aim of this study was to analyze the possible role of central administration of prolactin on spike-wave discharges in adult female taiep rats. We used 6-month-old female taiep rats with an implant of stainless-steel screw electrodes in the cerebral cortices using stereotaxic surgery. One week later, they were bilateral oophorectomized and maintain in permanent estrous using 17-β estradiol through s.c. injections in the neck region. EEG recordings of 8 h duration under control conditions and after i.c.v. injection of 0.5, 1 and 2 µg of prolactin/2 µL of artificial cerebrospinal fluid. All Ss were maintained under standard conditions and the experimental protocol was approved by IACUC. The results showed that prolactin did not increase the frequency of the absence seizures with the 3 doses tested ($P>0.05$). But prolactin increased the mean duration with 210% and 189%, with 0.5 and 1 µg doses, respectively ($P<0.001$). In fact, 2 µg of prolactin produced the highest increase in the duration of absence seizures 205% ($P<0.001$). In conclusion, prolactin is capable to change the activity of the thalamo-cortical circuit to sustain absence seizures in estrogenized female taiep rats.

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Poster

P147. Epilepsy and Seizures: Animal Models

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Topic: B.10. Epilepsy

Support: NIH grant R01 NS106688

Title: Ido2 deficiency is protective against acute seizures and hyperactivity in a kainic acid model of ictogenesis

Authors: *Z. A. MACDOWELL KASWAN, M. HURTADO, E. Y. CHEN, R. H. MCCUSKER;
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Abstract: Neuronal hyperexcitability, particularly in association with neuroinflammation, can trigger the development of acute seizures (ictogenesis), putting patients at risk of subsequent pathologies such as epilepsy and chronic behavioral changes. Neuroinflammation induces expression of indoleamine 2,3-dioxygenase (Ido) 1 and 2 within the central nervous system. The Ido's are the first and rate limiting enzymes of the kynurenine pathway, by which tryptophan is converted into kynurenine and subsequently into neuroactive metabolites. Kynurenine metabolites, some of which are ictogenic, directly interact with glutamate receptors in the brain to modulate neural activity. Ido1 and Ido2, therefore, may provide a mechanistic link between neuroinflammation and ictogenesis. In the kainic acid (KainA) model of ictogenesis, systemically administered KainA crosses the blood brain barrier to activate glutamate receptors and cause seizures. We used this model to explore the effect of Ido1 and Ido2 deficiencies on acute seizure susceptibility. Mice were injected intraperitoneally with five doses of 4.5-5 mg/kg KainA or saline control at intervals of 30 minutes and observed for seizures throughout the injection period and for an additional 30 minutes after the final dose. We found that mice deficient for Ido2 ($Ido2^{KO}$) are less likely to experience seizures following injection of KainA compared to wild-type (WT) C57BL/6 mice. In contrast, mice deficient for Ido1 ($Ido1^{KO}$) have equivalent seizure incidence compared to WT mice. $Ido2^{KO}$ mice are also protected from KainA-induced hyperactivity assessed 8 days post-injection. Gene expression was quantified in hippocampi collected 30 min after the final injection. Unexpectedly, hippocampal Ido1 and Ido2 expression is reduced by KainA treatment regardless of seizure incidence. However, KainA increased expression of the proinflammatory cytokines TNFα and IL-1β. Cytokine expression is higher in the hippocampi of mice that experienced seizures relative to those that did not, indicating higher levels of hippocampal neuroinflammation. Surprisingly, TNFα expression is higher in $Ido2^{KO}$ mice compared to WT or $Ido1^{KO}$ mice despite their lower seizure incidence. Our data indicate that the low seizure incidence of $Ido2^{KO}$ mice makes Ido2 a putative candidate for new anti-seizure therapeutics.

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Poster

P147. Epilepsy and Seizures: Animal Models

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Program #/Poster #: P147.03

Topic: B.10. Epilepsy

Support: NIH F31 NS120467

Title: Developmental breathing abnormalities in a Scn1a haploinsuffient model of DS

Authors: *B. MILLA[1], D. K. MULKEY[2];
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Abstract: Dravet Syndrome (DS) is a severe form of epilepsy with a high rate of sudden unexpected death in epilepsy (SUDEP). Respiratory failure is a leading cause of SUDEP, despite this it is unclear how DS-associated genetic variants or seizure activity in general disrupts respiratory control. Most DS cases are caused by mutations in the Scn1a gene, which encodes a voltage gated Na+ channel that preferentially regulates activity of inhibitory neurons. Consistent with this, we showed that loss of Scn1a function in inhibitory neurons disrupted CO2/H+-dependent regulation of breathing by the retrotrapezoid nucleus (i.e., RTN chemoreception), caused seizures and premature death. However, Scn1a is also expressed by excitatory neurons particularly later in development indicating loss of Scn1a function in excitatory neurons contributes to progression of DS symptoms. Therefore, the goal of this work is to determine whether and how respiratory function is perturbed across development in a global Scn1a haploinsuficient (Scn1a+/-) mouse model of DS. Scn1a+/- and littermate controls were maintained on 50:50 background of 129S1/SvlmJ::C57BL6/J. Respiratory activity was measured in the same animals at 13 and 21 days of age by whole-body plethysmography during exposure to room air, high CO2 (3-7% CO2) or low O2 (10% O2). At the cellular level, baseline activity and CO2/H+-sensitivity of RTN neurons in brain slices obtained from each genotype was characterized in cell-attached voltage clamp mode. Neonatal Scn1a+/- mice breathe normally in room air but show a blunted ventilatory response to graded increases in CO2. At the cellular level, RTN neurons in slices from Scn1a+/- mice show increased baseline activity compared to RTN neurons from control tissue. We also found the ventilatory response to CO2 improved in these same mice at a later developmental time point. However, breathing problems persisted in juvenile Scn1a+/- as a blunted ventilatory response to hypoxia and importantly this respiratory deficit positively correlated with mortality; mice that died by 24 days of age had a diminished hypoxic ventilatory response compared to Scn1a+/- that survived. Loss of Scn1a preferentially disrupts the CO2 ventilatory response at the cellular and whole animal levels early in development. These results are similar to the phenotype of mice with inhibitory neuron specific

Scn1a disruption, suggesting a dominate role of Scn1a in inhibitory neurons at this developmental time point. We also show that a blunted hypoxic ventilatory response correlates with premature death in juvenile Scn1a+/-. These results suggest multiple aspects of respiratory control are compromised in DS.

Disclosures: B. Milla: None. **D.K. Mulkey:** None.

Poster

P147. Epilepsy and Seizures: Animal Models

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P147.04

Topic: B.10. Epilepsy

Support: NIH Grant EB019804

Title: Tissue oxygenation dynamics in spontaneous seizure and seizure/spreading depolarization (SD) events in a rodent model of temporal lobe epilepsy

Authors: *J. LIU[1], B. J. GLUCKMAN[2];
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Abstract: Oxygen is critical in maintianing neuronal transmembrane ion gradients, and local deficits in oxygenation are known to be able to initiate spreading depolarization (SD). We demonstrated that SD frequently occurs associated with the seizures in the tetanus toxin rodent model of temporal lobe epilepsy (Bahari F 2020, https://doi.org/10.1101/455519). Here we investigate with continuous recordings if tissue oxygenation changes lead or follow the onset of seizures and the onset of seizure-associated SD.
Methods: We implemented long-term home-cage continuous tissue oxygenation and biopotential measurements on freely moving rats prepared under the tetanus toxin (TeTX) model of temporal lobe epilepsy (TLE) and applied both the constant potential amperometry (CPA) and long-term pulse voltammetry (LPV). Ideally, these methods yield measures of average tissue oxygen concentration C_0. CPA methods depend on both C_0 and average diffusion constant D_{eff} through tissue. In LPV, the initial peak current reflects the local C_0 while its tail is comparable to the mixed CPA measure. We introduce a method to estimate relative changes of D_{eff} from the LPV. We studied tissue oxygenation dynamics during the normal state of vigilance (SOV) transitions, peri-seizure transitions without SD (N=704/412 events), and peri-seizure transitions with SD (N=190/68 events) in (N=9/5) animals using (CPA/LPV) methods. Critically, these measures were done chronically during multi-day homecage recordings in freely behaving non-anesthetized animals.
Results: Across all events, we find comparable dynamics from both CPA and the tail portion of

LPV. For transitions between non-seizure SOV states, these dynamics are consistent with the peak LPV value, which best measures local C_0. This implies that D_{eff} doesn't change appreciably for transitions between non-seizures SOV states. Likewise, neither tissue oxygenation nor Deff changed appreciably ahead of seizure onset. Tissue oxygenation did change of order 5% during seizures and 20% during SD events. But no overt decreases were observed either during the pre-seizure period nor ahead of SD onset during seizures. Instead during seizures C_0 typically increased. In contrast, D_{eff} did decrease as much as 80% during SD events. Oxygen dynamics appear to follow seizure and SD onset transitions rather than precede and potentially cause them. Increases in tissue oxygenation during seizures indicate a robust neurovascular response. In addition, oxygen diffusion rates through tissue during SD are significantly decreased, presumably correlated with cell swelling and decreases extracellular space.

Disclosures: J. Liu: None. **B.J. Gluckman:** None.

Poster

P147. Epilepsy and Seizures: Animal Models

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Program #/Poster #: P147.05

Topic: B.10. Epilepsy

Support: Supported by Hearst Foundation American Physiological Society Summer Undergraduate Research Fellowship (to N.J.R.)
Nudelman Foundation Women in Neuroscience Summer Fellowship (to S.K.)

Title: Increased slow gamma power across behavioral states correlates with seizure phenotype severity in the Ank3-1b mouse model of epilepsy

Authors: J. E. VILLACRES PEREZ, N. J. RIVEIRA, S. KIM, L. L. COLGIN, *A. Y. LOPEZ;
Neurosci., Univ. of Texas At Austin, Austin, TX

Abstract: Absence epilepsy is characterized by spike-and-wave discharges (SWD) in electroencephalographic (EEG) signals that correspond to cognitive and behavioral arrest in patients. Many studies have identified various physiological effects of absence epilepsy on human development, and pharmaceutical intervention has significantly improved the treatment of absence seizures. Despite these advances, there remains little understanding of mood and behavioral comorbidities commonly present in pediatric and adult patients with childhood-onset absence epilepsy. The *Ank3* gene provides a unique opportunity to study these comorbidities, as it has been linked to mood disorders in human genetic studies and more recently to absence epilepsy in rodents. Thus, this study aims to identify unique event-related behavioral correlates in EEG signals of *Ank3-1b* knockout (KO) mice. Here, we take advantage of two supervised

learning algorithms to identify seizure events from EEG recordings and track animal movements from video recordings. Pose estimates were extracted from the video recordings to distinguish moving from nonmoving bouts. Then a binary classifier was developed to identify sleep states. Manually scored behaviors were used to generate the classifier's receiver operating characteristic curve and guide the choice of classification threshold. EEG signal power during these behavioral states was estimated for signal frequencies between 1 and 50 Hz using a Morlet wavelet transform. Preliminary results suggest that *Ank3-1b*$^{-/-}$ KO mice exhibit increased slow gamma (~30-40 Hz) power during sleep and moving bouts compared to wildtype controls. *Ank3-1b*$^{-/+}$ KO mice show an intermediate phenotype characterized by a relatively modest increase in slow gamma power. Preliminary results also suggest that these increases in slow gamma power correlated with gene dosage and seizure phenotype severity. These results provide a foundation for exploring gamma oscillations during complex behaviors related to mood disorders in *Ank3* mouse models of epilepsy.

Disclosures: J.E. Villacres Perez: None. **N.J. Riveira:** None. **S. Kim:** None. **L.L. Colgin:** None. **A.Y. Lopez:** None.

Poster

P147. Epilepsy and Seizures: Animal Models

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Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P147.06

Topic: B.10. Epilepsy

Support: NS107148
NS079507

Title: A home-cage monitoring solution for non-invasive seizure screening in rodent models of epilepsy

Authors: D. M. HUFFMAN[1], P. RUKUNDO[2], D. IRADUKUNDA[2], J. PERDEH[3], K. DONOHUE[1], B. F. O'HARA[4], B. BAUER[3], S. SUNDERAM[2];
[1]Signal Solutions, LLC, Lexington, KY; [2]F. Joseph Halcomb III, M.D. Dept. of Biomed. Engin., [3]Dept. of Pharmaceut. Sci., [4]Dept. of Biol., Univ. of Kentucky, Lexington, KY

Abstract: Rationale: There is an urgent need for research into treatment options for epilepsy and other seizure disorders. Animal models are increasingly used to understand disease mechanisms and to screen potential therapeutic approaches. However, such investigations typically require validation via expensive and labor-intensive methods (i.e., EEG measurements), limiting the pace and scale of experimentation. Previously, we have shown that piezoelectric technologies have great utility in non-invasive seizure detection. We present further developments in this effort where monitoring is accomplished through an external sensor

platform located beneath the housing cage.**Methods:** Adult Wistar rats (female; 2 months) were treated with pilocarpine i.p. to induce status epilepticus (SE). Three months after SE, rats were monitored individually in standard cages via piezoelectric *Adapt-a-Base* sensors (Signal Solutions, LLC) alongside a continuous video record for 10 weeks. Weekly recordings were processed to derive a seizure detection statistic time series, to which a threshold was applied to identify the 20 most intense events (20 events/week × 8 cages = 160 events/week). Timestamps of threshold crossings were saved to a spreadsheet for inspection through the video record, and were annotated according to observed behavior (i.e., seizure, grooming, etc). Detection precision was evaluated according to the incidence of true positive detections relative to all detections. **Results:** Piezoelectric sensors captured the essential dynamics of seizures, as well as pre- to post-ictal changes in physiological parameters (i.e., breathing). Derivation of a simple detection statistic was successful in discriminating seizure events, albeit with variable precision (5-65%); likely related to the variability in true seizure yield. **Conclusions:** Overall, this approach shows great utility in identifying spontaneous seizures in chronic epilepsy monitoring; saving labor in identifying seizures compared to exhaustive video review. Piezoelectric signals captured tonic-clonic behavior during seizures and other relevant behaviors such as breathing, freezing, and tremor. Moreover, as this technology has been validated for use in discriminating sleep-wake activity, it opens the door to investigating seizures in the context of sleep/circadian rhythms, which may be a key aspect to consider when evaluating potential treatment strategies. Further development of this method will focus on capturing the temporal evolution of the piezo signal during seizures, aimed at increasing detection sensitivity/specificity, as well as discriminating seizure severity.

Disclosures: D.M. Huffman: A. Employment/Salary (full or part-time):; Signal Solutions, LLC. **P. Rukundo:** None. **D. Iradukunda:** None. **J. Perdeh:** None. **K. Donohue:** A. Employment/Salary (full or part-time):; Signal Solutions, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Signal Solutions, LLC. **B.F. O'Hara:** A. Employment/Salary (full or part-time):; Signal Solutions, LLC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Signal Solutions, LLC. **B. Bauer:** None. **S. Sunderam:** None.

Poster

P147. Epilepsy and Seizures: Animal Models

Location: Virtual Only

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Program #/Poster #: P147.07

Topic: B.10. Epilepsy

Support: NIH R01 NS066974

Title: Locus coeruleus neuronal activity in an awake mouse model of temporal lobe seizures investigated by juxtacellular recordings

Authors: *M. VALCARCE-ASPEGREN, Q. WU, L.-A. SIEU, P. PASZKOWSKI, A. SHARAFELDIN, H. BLUMENFELD;
Dept. of Neurol., Yale Sch. of Med., New Haven, CT

Abstract: Inhibition of brainstem and basal forebrain cholinergic neurons has been identified in previous rat models as a potential driver of decreased arousal during temporal lobe seizures. The subcortical pathways modulating depressed cortical function during focal limbic seizures remains largely unclear. Recording single-cell activity in the locus coeruleus in an awake mouse model may help elucidate these pathways by examining ictal noradrenergic modulation. Additionally, these studies can show the plausibility of ictal juxtacellular recording of individual neurons in the deep brainstems of awake, moving mice, allowing for future study of other specific subcortical regions of interest. Recordings were performed using head-fixed mice running on a wheel. Chronically implanted bipolar electrodes were used to measure local field potentials in the right orbitofrontal cortex and bilateral hippocampi. Focal limbic seizures were induced via the application of current pulses (2s, 60 Hz) into the hippocampus. Noradrenergic neurons in the locus coeruleus were recorded using 1.5mm glass capillaries filled with 4% Neurobiotin. Neuronal identification was histologically achieved through double staining with cy3-streptavidin and anti-tyrosine hydroxylase antibodies. It was possible to both find and maintain a stable signal from single neurons deep in the brainstem of moving animals (n=9). Preliminary data indicate decreased firing frequency and changes in firing patterns of individual neurons in the locus coeruleus during focal hippocampal seizures. Histology successfully identified labeled neurons in the locus coeruleus. In summary, we found that juxtacellular recordings of individual neurons in the deep brainstem is feasible in awake, moving mice during seizures. Additionally, changes in the firing patterns of neurons in the locus coeruleus appear to have a potential role in changing arousal during focal limbic seizures. To further uncover the mechanisms underlying ictal unconsciousness it is critical to continue exploring specific subcortical neurons and neurotransmitters affected during seizures. Increased understanding of these processes may in turn lead to beneficial, novel treatments for people with epilepsy.

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Poster

P147. Epilepsy and Seizures: Animal Models

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Program #/Poster #: P147.08

Topic: B.10. Epilepsy

Title: Viral activation of the medial septal nucleus improves spatial memory and increases seizure threshold in a pilocarpine model of status epilepticus

Authors: N. C. ZEPEDA, S. MEDVIDOVIC, M. BERGOSH, W. CHOI, *D. J. LEE;
USC, Los Angeles, CA

Abstract: Recently thalamic deep brain stimulation (DBS) and responsive neurostimulation have been approved for the treatment of medically refractory epilepsy; however, the underlying mechanism of electrical stimulation remains largely unknown. We have previously demonstrated that hippocampal theta power is diminished in animal models of epilepsy with concomitant with deficits in spatial learning. Data from our lab, demonstrates that MSN theta DBS can increase hippocampal oscillations and restore cognitive function in a rodent epilepsy model. In this study, we are using a chemogenetic approach to drive the septohippocampal circuit to increase seizure threshold. Methods: 32 male Sprague-Dawley rats were divided into animals undergoing a pilocarpine injection to induced status epilepticus (n=20) or saline vehicle injection (n=12). 13 days after injection, seizure threshold was measured using flurothyl testing. The following day rats underwent unilateral implantation of a hippocampal recording electrodes as well as bilateral AAV8 intracranial injection of either AAV8-CamKIIa-hM3D(Gq)-mcherry or AAV8-CamKIIa-EGFP (sham) into the medial septal nucleus. On post-injection days 21-25, animals underwent the Barnes maze spatial memory task and given an intraperitoneal injection of either clozapine N-oxide to active the receptor or saline 25 minutes prior to the task. On post-injection day 29, animals were again administered clozapine N-oxide or saline. 25-minutes after injection, repeat flurothyl testing was performed. Results: Pilocarpine-injected rats (sham activation) had a longer latency to finding the escape hole compared to saline-injected rats ($p<0.05$). Pilocarpine-injected rats with MSN activation (AAV8-CamKIIa-hM3D(Gq)-mcherry) had a shorter latency than sham activation pilocarpine-injected rats ($p<0.05$). Latency was similar in saline-injected rats and MSN activated pilocarpine rats. Pilocarpine-injected rats (sham activation) had a lower seizure threshold compared to saline-injected rats (299.6±13.8 seconds vs. 405.6±34.0; $p<0.05$). Pilocarpine-injected rats with MSN activation had a higher seizure threshold compared to sham activation pilocarpine-injected rats (400.2±34.0; $p<0.05$). Conclusion: Viral activation of the medial septal nucleus increases seizure threshold and improves spatial memory in pilocarpine-induced status epilepticus rats.

Disclosures: N.C. Zepeda: None. **S. Medvidovic:** None. **M. Bergosh:** None. **W. Choi:** None. **D.J. Lee:** None.

Poster

P147. Epilepsy and Seizures: Animal Models

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Topic: B.10. Epilepsy

Support: NIH R01 NS066974
R01 NS096088
T32 NS007224

Title: Cortical slow waves and reduced cholinergic arousal in novel mouse model of focal limbic seizures with impaired behavior

Authors: *L.-A. SIEU[1], S. SINGLA[1], A. SHARAFELDIN[1], G. CHANDRASEKARAN[2], M. VALCARCE-ASPEGREN[1], A. NIKNAHAD[3], I. FU[1], N. DOILICHO[1], A. GUMMADAVELLI[1], C. MCCAFFERTY[4], R. B. CROUSE[1], Q. PERRENOUD[1], M. R. PICCIOTTO[1], J. CARDIN[1], H. BLUMENFELD[1];
[1]Yale Sch. of Med., New Haven, CT; [2]Univ. of Pennsylvania, Philadelphia, PA; [3]John Hopkins school of medicine, Baltimore, MD; [4]Univ. Col. Cork, Cork, Ireland

Abstract: Temporal lobe epilepsy (TLE) is often associated with loss of consciousness and therefore reduced patient quality of life. Clinical studies have shown that this loss of consciousness is positively correlated with neocortical slow waves on EEG, similar to the non-REM sleep state. Further investigation in the rat model of focal limbic seizures revealed that subcortical arousal systems are depressed during seizures, including brainstem and basal forebrain cholinergic neurons. To better elucidate underlying circuit and network mechanisms resulting in loss of consciousness, we developed an awake, behaving mouse model of focal limbic seizure to take advantage of the wealth of genetics tools available. Focal limbic seizures were recorded and unilaterally induced by a 2s, 60 Hz hippocampal stimulus via implanted bipolar electrodes in the dorsal hippocampus, while local field potential (LFP) signals were recorded simultaneously from orbitofrontal cortex (OFC). To assess behavioral responses during seizures, water-restricted mice were trained to lick a water dispenser in response to a sound (0-50kHz noise, 12ms) every 10-15s while head-fixed on a running wheel. To characterize cortical slow waves, multiunit activity (MUA) was recorded in left OFC via a tungsten electrode. Cholinergic input to OFC during seizures was investigated by expressing a genetically encoded fluorescent acetylcholine indicator (GACh) in the OFC and signals were detected via an implanted optic fiber into OFC (fiber photometry). Focal seizures were inducible for several weeks and were of 5-30s duration (n=20 animals). Overall responses to sound and running speed decreased during seizures and recovered post-ictally ($p<0.01$, n=20 animals). However, responses to sound were sometimes normal during seizures, similar to TLE in humans. By examining behavioral responses and cortical activity, we showed that behavioral arrest is correlated with amplitude of cortical slow-wave activity. Cortical slow waves followed an Up and Down state firing pattern on MUA recordings, like the slow wave sleep (n=6 animals). Further, cholinergic input to the cortex was reduced during seizures, with larger decreases associated with impaired behavior (n=5 animals). These results suggest this novel mouse model of focal limbic seizures shares characteristics with the rat model and human temporal lobe seizures. Impaired consciousness is associated with depressed cortical arousal facilitated by reduced cholinergic input. Future work with this model will help to uncover fundamental mechanisms for loss of consciousness in focal seizures in hope to lead to innovative treatment.

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Poster

P150. Antiepileptic Therapies II

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Program #/Poster #: P150.01

Topic: B.10. Epilepsy

Support: H2020-MSCA-ITN-2016

Title: Maturation of hESC-derived GABAergic interneurons in epileptic rodent hippocampus

Authors: *E. WALOSCHKOVÁ, A. GONZALEZ-RAMOS, A. MIKROULIS, M. LEDRI, M. ANDERSSON, M. KOKAIA;
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Abstract: Epilepsy is a complex disorder affecting the central nervous system and is characterised by uncontrollable spontaneously recurring seizures. Patients suffering from epilepsy are treated with various anti-seizure medication, however, this is ineffective in 30%, mostly in patients suffering from temporal lobe epilepsy (TLE). Therefore, there is a need for developing novel treatment strategies. One of these novel approaches is transplantation of cells releasing gamma-aminobutyric acid (GABA) to restore the balance between excitation and inhibition within the neuronal network. We generated optogenetically modified GABAergic interneuron precursors from human embryonic stem cells (hESCs) by overexpressing two transcription factors, *Ascl1* and *Dlx2*, and transplanted them in the hippocampi of a rodent TLE model. For establishing the model, 8-week-old male Rowett Nude (RNU) rats were treated with a titration-based kainic acid injection protocol until reaching status epilepticus (SE) and behavioural seizures were confirmed by video monitoring 3 months later. By performing whole-cell patch-clamp recordings in slices from the transplanted epileptic animals, we thoroughly characterised the maturation of the transplanted cells into functional GABAergic interneurons. Furthermore, by utilising their optogenetic modification and by using picrotoxin (a $GABA_A$ receptor blocker) we confirmed the presence of functional inhibitory synaptic connections from the transplanted cells onto the host neurons both 3 months (n=50 cells, 7 animals) and 6 months post-transplantation (n=78 cells, 8 animals). Moreover, we fixed and sub-sliced the tissue collected after electrophysiological experiments and performed immunohistochemistry on the slices. We confirmed the expression of calbindin and calretinin (two main interneuron markers expressed when using the *in vitro* differentiation protocol) in the transplanted cells as well as release of GABA from their efferent synaptic connections. In addition, we observed a tendency

for reduced seizure frequency in transplanted animals compared to untreated controls at 4 months post-SE. In summary, these data present a proof-of-concept for a cell-based therapeutic approach in treating epilepsy.

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Poster

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Topic: B.10. Epilepsy

Support: R01NS097802
Epilepsy Foundation
FSU college of medicine

Title: Intervention in the Medial Entorhinal Area recues TLE-related pathology in CA1 hippocampus via the temporoammonic pathway.

Authors: *S. BEESLEY[1], T. SULLENBERGER[2], S. S. KUMAR[3];
[1]Florida State Univ., Tallahassee, FL; [2]Florida State Univ., tallahassee, FL; [3]Biomed. Sci., Florida State Univ., Tallahassee, FL

Abstract: Temporal lobe epilepsy (TLE) is the most common form of drug-resistant epilepsy in adults, with an unknown etiology. A hallmark of TLE is the characteristic loss of layer 3 neurons in the medial entorhinal area (MEA) that underlies seizure development. The MEA is known to entrain the hippocampus primarily through the perforant pathway (PP), which connects stellate cells in layer 2 of the MEA with granule cells of the dentate gyrus to drive the hippocampal tri-synaptic circuit. Using immunohistochemistry, high-resolution confocal microscopy, and the rat pilocarpine model of TLE, we show here that the lesser known temporoammonic pathway (TAP) plays a significant role in transferring MEA pathology to the CA1 region of the hippocampus independently of the PP. Our previous studies revealed that daily intracranial infusion of D-serine (100 μM), an antagonist of GluN3-containing triheteromeric N-Methyl D-aspartate receptors (t-NMDARs), into the MEA prevented loss of layer 3 neurons and curtailed subsequent epileptogenesis. This intervention in the MEA also led to the rescue of hippocampal CA1 neurons, and the down regulation of astrocytes and microglia expression, thereby mitigating the effects of neuroinflammation. Interestingly, these changes were not observed to a similar extent in other regions of vulnerability like the hilus, DG or CA3, suggesting that the pathology manifest in CA1 is driven predominantly through the TAP. These findings aid in better

understanding the factors driving hippocampal pathophysiology and are of relevance in seeking therapeutic avenues to mitigate the cognitive co-morbidities associated with this disease.

Disclosures: S. Beesley: None. **T. Sullenberger:** None. **S.S. Kumar:** None.

Poster

P150. Antiepileptic Therapies II

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Program #/Poster #: P150.03

Topic: B.10. Epilepsy

Support: NIH Grant R01NS092705 (to CG)

Title: The effect of antagonism of miR-324-5p on epileptogenesis following intrahippocampal kainic acid injections in mice.

Authors: *A. MCGANN[1,2], D. TIWARI[1,3], C. GROSS[1,3];
[1]Neurol., Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH; [2]Neurosci. Grad. Program, Univ. of Cincinnati Col. of Med., Cincinnati, OH; [3]Pediatrics, Cincinnati Children's Hosp., Cincinnati, OH

Abstract: Epilepsy affects over 50 million people worldwide. It is often caused by an initial brain insult, such as traumatic brain injury or infection, that is followed by the development of spontaneous recurrent seizures. The process by which the brain becomes prone to spontaneous recurrent seizures following an initial insult is termed epileptogenesis. The details of this process are still largely unknown, and further elucidation of the molecular and cellular mechanisms turning a healthy brain into an epileptic brain is needed to develop treatment strategies to prevent epileptogenesis. MicroRNAs have emerged as critical regulators of neuronal excitability via regulation of mRNA translation and stability, and are often altered in epilepsy. Our lab has shown that miR-324-5p is a critical regulator of neuronal excitability that acts by inhibiting expression of the A-type potassium-channel Kv4.2. Antagonism of miR-324-5p decreases seizure susceptibility and reduces seizure frequency in chronically seizing mice. It remains unknown, however, whether antagonism of miR-324-5p shortly following a brain insult can inhibit the progression of epileptogenesis. Here, we tested the hypothesis that miR-324-5p contributes to the development of epilepsy following *status epilepticus*. We used the intrahippocampal kainic acid model to induce epileptogenesis in wild type male mice aged 6-8 weeks. Twenty-four hours after kainic acid-induced *status epilepticus*, we administered a miR-324-5p or scrambled antagomir intracerebroventricularly and implanted cortical surface electrodes for EEG monitoring. EEG data was collected for a period of 28 days, during which the majority of mice developed spontaneous seizures. Mouse brain tissue was then perfused for assessment of neuronal cell death. Data was collected for 6 mice per group. Researchers were

blinded to treatment condition during surgery, EEG analysis, and histological analysis. EEG data was assessed for time to seizure onset, frequency, and duration, as well as interictal spike activity. Results indicated high levels of variability within treatment groups requiring larger sample sizes to determine potential effects. Ongoing work in the lab aims to increase sample sizes and clarify the network of mRNAs targeted by miR-324-5p to identify the critical genes controlling epileptogenesis. Many miRNAs have been implicated in brain hyperexcitability and it is likely that antagonism of miR-324-5p alone is insufficient to result in complete inhibition of epileptogenesis. Future work is needed to assess whether combined inhibition of several epileptogenic microRNAs leads to more complete prevention of epileptogenesis.

Disclosures: A. McGann: None. **D. Tiwari:** None. **C. Gross:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); C.G. is co-Inventor on US patent 9,932,585 B2..

Poster

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Program #/Poster #: P150.04

Topic: B.10. Epilepsy

Support: NINDS 1F31NS122484-01 to Kraus, K.L.

Title: Bimodal regulation of status epilepticus by hippocampal glucocorticoid receptor deletion

Authors: *K. L. KRAUS[1,3], C. M. GODALE[3], A. CHORDIA[3], N. NAWREEN[2], C. L. LASARGE[3], J. P. HERMAN[2], S. C. DANZER[3];
[1]Med. Scientist Training Program, [2]Dept Pharmacol. and Systems Physiol., Univ. of Cincinnati, Cincinnati, OH; [3]Dept of Anesthesia, Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH

Abstract: Status epilepticus (SE) is a life-threatening medical emergency defined by sustained seizure activity. Current treatments fail to terminate SE in all patients, highlighting a critical need to better understand the mechanisms driving SE severity and duration. Seizures are associated with a rapid increase in glucocorticoid levels, which can modulate neuronal excitability in the hippocampus through the activation of glucocorticoid (GRs). Previous studies suggest that corticosterone (CORT) exposure can be pro-convulsant and that GR antagonism after SE can reduce hippocampal cell death. However, with widespread expression across the brain and body, the precise role of GRs in the hippocampus during SE is unclear. Here, we tested the hypothesis that GR deletion from ventral hippocampal glutamatergic neurons will decrease SE severity. To delete GRs, adult male and female $GR^{fl/fl}$ (B6.Cg-*Nr3c1*$^{tm1.1Jda}$/J) mice on a C57BL/6 background underwent bilateral stereotaxic injection of AAV9.CaMKII.Cre-mCherry into the ventral hippocampus (GRKO, N = 23). Controls included $GR^{fl/fl}$ mice injected with AAV9.CaMKI-

mCherry and $GR^{wt/wt}$ mice injected with the Cre-mcherry virus (Control, N=25). Two weeks later, mice received pilocarpine (400 mg/kg, s.c.) to induce SE. GR deletion was confirmed with immunohistochemistry and SE severity was determined with modified Racine scale behavioral scores. Survivors received diazepam at 75-min post-pilocarpine and were sacrificed 48hrs post-SE. GR deletion primarily affected hippocampal dentate granule cells, mossy cells, and variable numbers of CA1, CA3 and subicular pyramidal neurons. Latency to Racine class III partial seizures after pilocarpine injection was reduced among GRKO mice. Paradoxically, latencies to both class IV and V convulsive seizures were increased among GRKO mice. Although overall mortality was high in both groups (Con: 85%, GRKO: 65%), GR deletion significantly delayed the onset of death. Baseline morning corticosterone (CORT) concentrations were not impacted by GRKO. Measures of serum CORT revealed a rapid increase after the onset of SE; however, increases were similar between control and GRKO mice. In conclusion, GR deletion from ventral hippocampal glutamatergic neurons had divergent effects on early vs late seizure pathology during SE, which may reflect the state-dependent action of hippocampal GRs. These preclinical data shed light on the seizure mechanisms which are stress-dependent, building the rationale for the exploration of GR-modulating drugs as potential adjunctive treatments for late SE.

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Poster

P150. Antiepileptic Therapies II

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Program #/Poster #: P150.05

Topic: B.10. Epilepsy

Support: NIH/NINDS R01NS115800
Iowa Neuroscience Institute

Title: Lacosamide decreases seizure-like activity in the neocortex of neonatal mice

Authors: *R. L. LANGTON, J. GLYKYS;
Pediatrics, Univ. of Iowa Carver Col. of Med., Iowa City, IA

Abstract: A high proportion of neonates experiencing seizures fail to respond to first-line anticonvulsant medications. Phenobarbital, a $GABA_A$ receptor allosteric modulator, has low efficacy in a significant number of neonatal seizures, yet it is one of the most used anticonvulsants in this age group. In mice, the poor effectiveness of phenobarbital is due to a higher intraneuronal chloride concentration in the neonatal neocortex compared to the adult one, resulting in more depolarizing actions of GABA. One approach to treat neonatal seizures is to

use anticonvulsants that do not rely on GABAergic modulation. Therefore, we investigated if lacosamide, a non-GABAergic anticonvulsive drug, decreases seizure-like activity in the neonatal neocortex *in vitro*. We tested the effect of different concentrations of lacosamide on seizure-like activity induced by the pro-convulsant drug 4-aminopyridine in adult (1-1.6 month-old) and neonatal (postnatal day 8-11) neocortical slices (layer IV/V) from wild-type C57BL/6J mice. We observed that lacosamide decreased seizure-like events in the neocortex of both adult and neonatal slices in a concentration-dependent manner. The ratio of seizures (events longer than 10 seconds) to total events also significantly decreased with lacosamide in both neonates and adults. We conclude that lacosamide effectively reduces seizure-like activity in neonates with similar efficacy as in adult brain slices. Our results support the rational use of lacosamide in the treatment of neonatal seizures.

Disclosures: R.L. Langton: None. **J. Glykys:** None.

Poster

P150. Antiepileptic Therapies II

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P150.06

Topic: B.10. Epilepsy

Support: Swedish Research Council Grant 2016-02605
Swedish Brain Foundation
European Union Horizon 2020 Programme (H2020-MSCA-ITN-2016) under the Marie Skłodowska-Curie Innovative Training Network, project Training4CRM Grant Agreement No. 722779

Title: Human stem cell-derived interneurons transplanted into a genetic mouse model of cortical dysplasia-focal epilepsy syndrome

Authors: *A. GONZALEZ-RAMOS, F. BERGLIND, A. MIKROULIS, K. LAURIN, D. HATAMIAN, M. HAYATLEH, A. VOMBERGAR, A. BOSIO, M. LEDRI, M. KOKAIA, M. S. ANDERSSON;
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Abstract: Focal cortical dysplasia (FCD) is a group of developmental malformations of the cortex manifesting as confined regions of pathology. It is the most common cause of drug resistant epilepsy in children, with an estimated half of children undergoing epilepsy surgery diagnosed with FCD as well as a fifth of adults, creating a need for new therapeutic strategies. Previous studies have shown that early postnatal transplants of allogenic embryonic medial ganglionic eminence precursor cells or postinduction transplantation of human GABAergic neuronal progenitors enhances inhibition and decreases seizures in diverse rodent models of

temporal lobe epilepsy. Based on these positive results, we have explored a GABAergic cell-based replacement therapy for cortical dysplasia-focal epilepsy syndrome using the *Cntnap2*$^{-/-}$ mouse model, which develops spontaneous reoccurring seizures at six months of age. GABAergic neurons mainly expressing Calretinin and Calbindin were generated from human embryonic stem cells (H1 cell line) using transient expression of *Ascl1* and *Dlx2*. Human embryonic stem cell-derived interneurons (hdIN) were transplanted unilaterally to dorsal hippocampus of postnatal day 2 *Cntnap2*$^{-/-}$ mice and behavioral tests assessing memory function, cognitive flexibility, anxiety, and locomotor activity were performed at 4 and 6 months of age, followed by 2 weeks of continuous video-EEG monitoring (cell group n= 32, and sham group n=26). Histological evaluation confirmed survival of hdIN in all time points studied, up to 9 months, with hdIN migrating to contralateral hippocampus and adjacent structures. Behavioral seizures were significantly reduced in the cell- compared to the sham-treated group suggesting that hdIN transplantation reduces the severity of seizures but does not prevent the development of epilepsy in this model.

Disclosures: A. Gonzalez-Ramos: None. **F. Berglind:** None. **A. Mikroulis:** None. **K. Laurin:** None. **D. Hatamian:** None. **M. Hayatleh:** None. **A. Vombergar:** None. **A. Bosio:** None. **M. Ledri:** None. **M. Kokaia:** None. **M.S. Andersson:** None.

Poster

P199. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches VIII

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P199.01

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

Support: NIH/NIA R01 AG057008
NIH/NIA R01 AG058068
NIH/NIA UH2/3 NS100127
Administrative supplement to R01 AG057008
Philanthropic support from Lou and Christine Friedrich

Title: Age, APOE and sex modify the bacterial composition of the gut microbiome in EFAD transgenic mice

Authors: *J. E. MALDONADO WENG[1], J. GAWENDA[1], A. AAMIR[1], Z. ISLAM[1], J. YORK[1], S. J. GREEN[2], M. LADU[1];
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Abstract: The gut microbiome (GM) is a modifiable metabolic organ composed of gastrointestinal bacteria, emerging as an important component of Alzheimer’s disease (AD) pathology. AD patients exhibit GM dysbiosis, a decrease in beneficial bacteria with an increase

in toxic microbial species. This disrupts the digestion of carbohydrates, lipids and proteins, limiting the production of crucial metabolites. However, it remains unclear how the universal biological variables (UBVs) of AD risk (age, *APOE* genotype, and sex) affect the GM bacterial composition. While age is the greatest risk factor for AD, *APOE4* as the greatest genetic risk factor, and females (♀) exhibit a 2-fold greater risk than males (♂). To study the interactions among UBVs, we utilized the EFAD transgenic mice (5xFAD$^{+/-}$/*APOE*$^{+/+}$), a mouse model that exhibits an age-dependent AD-like phenotype that includes increased behavioral deficits, Aβ deposition and neuroinflammation, with the following hierarchy: ♂E3FAD < ♀E3FAD = ♂E4FAD < ♀E4FAD. Our previous analysis of fecal samples from 4-month (M) ♂ and ♀ E3FAD and E4FAD mice demonstrated dysbiosis, specifically decreased abundance and richness in the GM, that reflected the hierarchy observed with the other measures of pathology, ♂E3FAD < ♀E3FAD = ♂E4FAD < ♀E4FAD. The current study addresses the effects of age by generating and comparing the compositional profiles, across taxonomic levels (amplicon sequence variants; ASVs, Genus, and Family), of the GM in 4M and 8M, ♂ and ♀, E3FAD and E4FAD mice. **Thus, our hypothesis is that the distinct GM compositional profiles will reflect an age-associated increase in AD pathology in EFAD mice.** Using 16S rRNA amplicon sequencing, abundance and richness were compared across samples via permutational analysis of variance (PERMANOVA). Analysis of similarities (ANOSIM) quantified the magnitude of differences in diversity with an R value that ranges 0 - 1, where 1 is complete dissimilarity. The results demonstrated that age increased the *APOE* and sex-driven differences of the compositional profiles. An unbiased heatmap analysis of 60 ASVs, identified by a machine-learning algorithm, distinguished 4M and 8M cohorts and clustered samples following the hierarchy of AD pathology we have established in the EFAD mice. Notably, PERMANOVA demonstrated that sex played a greater role than *APOE* in defining the composition profiles at 8M; inversely, the role of *APOE* was greater at 4M. This indicates that the role of *APOE* and sex in shaping the GM is dependent on age. Overall, distinguishing the GM of ♂E3FAD, ♀E3FAD, ♂E4FAD and ♀E4FAD across age is critical step in establishing the functional metabolic role of the GM in the context of AD pathology.

Disclosures: J.E. Maldonado Weng: None. **J. Gawenda:** None. **A. Aamir:** None. **Z. Islam:** None. **J. York:** None. **S.J. Green:** None. **M. LaDu:** None.

Poster

P199. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches VIII

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P199.02

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

Support: T32AA025606
P50 AA017823

P50 AA017823-S1

Title: Emergence of age-specific spatial memory deficits in the Transgenic Fischer 344 rodent model of Alzheimer's Disease (TgF344-AD) following adolescent intermittent ethanol exposure

Authors: *N. L. REITZ, A. J. STANWISE, P. MARSLAND, T. DEAK, L. M. SAVAGE; Behavioral Neurosci. Program, Dept. of Psychology, Binghamton University-SUNY, Binghamton, NY

Abstract: Alcohol-related brain damage (ARBD) and Alzheimer's disease (AD) have considerable overlap in key neuropathologies. One of the most consistent pathologies throughout the progression of AD and ARBD is the loss of cholinergic projections, largely from the basal forebrain to the hippocampus and associated cortices. By utilizing different aging timepoints (3- and 6-months) that encompass prodromal AD in rodent models, we tracked developmental ethanol-induced alterations in disease progression as determined by a cognitive battery that was assessed in a longitudinal design. The current study characterized spatial and social memory deficits in a transgenic rat model of AD (TgF344-AD) following adolescent intermittent ethanol (AIE) exposure. TgF344-AD and wildtype F344 rats (male and female) were exposed to an intragastric gavage of water (control) or 5g/kg of 20% ethanol (AIE) for a 2 day on/off schedule from PD27-57. AIE-exposed rats reached binge-like levels of ethanol intoxication as measured by their blood ethanol concentration (BEC; ~201 mg/dl). There were no differences in BECs between genotype or sex. Rats of both genotype and exposure groups gained weight at similar rates (separated by sex) throughout the AIE-exposure period. Following AIE, all rats completed a spontaneous alternation task on an elevated plus maze at PD90 (3 months) and PD180 (6 months). Overall, TgF344-AD rats had impaired spatial working memory when compared to wildtype rats at both 3- and 6-months of age. At 3- and 6-months of age, AIE-exposed TgF344-AD rats had significantly lower percent alternations when compared to both control and AIE-exposed wildtype rodents. However, AIE-exposed TgF344-AD rats were the only group that showed a further deficit in spatial memory at 6-months, relative to their 3-month scores. This indicates that AIE is accelerating the emergence of memory impairments in a rodent model of AD as early as 6 months of age. Behavioral data from a final aging timepoint (9 months) will be discussed in comparison to the 3- and 6-month data. Despite the lack of sex differences so far, we will continue to monitor sex as a variable through 9 months as AD prevalence and symptom progression has been shown to be sexually dimorphic in both pre-clinical and clinical settings.

Disclosures: N.L. Reitz: None. **A.J. Stanwise:** None. **P. Marsland:** None. **T. Deak:** None. **L.M. Savage:** None.

Poster

P199. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches VIII

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P199.03

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

Support: NIH R01 AG060148
NIH T32 AG000096
Genomic High Throughput Facility, UC Irvine
UCI Microbiome Consortium

Title: Housing Conditions Influence Mouse Fecal Microbiome Composition of C5aR1KO in the Arctic AD mouse model

Authors: *T. PETRISKO[1], S.-H. CHU[1], P. SELVAN[1], K. L. WHITESON[1], A. J. TENNER[2]; [1]Univ. of California, Irvine, CA; [2]Univ. California Irvine, Irvine, CA

Abstract: The complement (C') system, a critical component of the innate immune system, is activated in the context of Alzheimer's disease (AD) pathology. Overactivation of the C' cascade in AD is hypothesized to result in enhanced inflammation, synapse loss and cognitive decline. Previous studies have demonstrated that genetic ablation or pharmacologic inhibition of the downstream C5a receptor C5aR1, provides protection from gliosis and cognitive decline in AD animal models, although the underlying mechanisms remain unclear. The C' system is expressed throughout the body, including in the gut, and as a result may influence the composition of the gut microbiome. Importantly, gut microbes influence an animals' behavior, including cognitive changes via the gut-brain-axis. The objective of this study was to determine if constitutive deletion of C5aR1 (C5aR1KO) alters the fecal microbiome in either WT or the Arctic (Arc) model of AD, and if these changes could contribute to observed changes in AD-associated behavior. Due to the strong effect of microbiome normalization due to the coprophagic activity of mice, we also sought to determine the impact on the microbiome when C5aR1KO and C5aR1-suffcient (C5aR1+/+) were housed separately or together. Fecal samples were collected at 10 mo, and subjected to microbial focused Illumina sequencing.When housed separately from C5aR1+/+ mice, WT C5aR1KO animals had a significantly reduced alpha-diversity, ie. number of species within their microbiome, as measured by the Shannon Diversity Index, compared to C5aR1+/+ mice. Arc C5aR1KO mice had a trending decrease ($p<0.09$) compared to their Arc controls. Beta-diversity, as calculated by Bray-Curtis dissimilarities, indicated significant variation between the genotypes, and further analysis revealed WT C5aR1+/+ mice had increased levels of the genus *Alistipes* compared to both WT/Arc C5aR1KO mice, while WT C5aR1KO had elevated levels of *Turicibacter* compared to WT and Arc animals. In contrast, when all 4 genotypes were housed together, no difference in alpha- or beta- diversity was observe. Cohousing also eliminated differences in *Alistipes* and *Turicibacter* levels. This study demonstrates that deletion of C5aR1 does influence the fecal microbiome, but only when KO mice are housed alone. In Hernandez et al, 2017, in which C5aR1KO mice were housed separately, Arc mice displayed reduced cognition compared to WT. As there was no difference in the microbiome of WT and Arc C5aR1+/+ animals, cognitive changes in these animals were not due to different populations of microbes. However, cohousing between all genotypes is required to fully eliminate microbiome from CNS causal effects.

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Poster

P199. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches VIII

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P199.04

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

Support: NIH AG062968
NIH P30AG013854

Title: Expression of sex hormone receptors differs in postmortem brain regions between Alzheimer's Disease subjects with and without prior depression

Authors: *K. PRZYBYL[1], W. LUO[1], E. H. BIGIO[2], S. WEINTRAUB[2], M.-M. MESULAM[2], E. E. REDEI[1];
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Abstract: Stress-related disorders, like depression, has been linked to a state of accelerated aging and subsequent pathological cognitive aging. Major depressive disorder (MDD) is a risk factor for the development of dementia, and MDD prevalence rate is up to 37% in dementia. Even moderate depression increases risk of progression from healthy to mild cognitive impairment, and to dementia. Prevalence of both MDD and dementia are higher in women compared to age-matched men. Progression of cognitive decline and its association with MDD shows that females are particularly vulnerable. Men and women with AD exhibit different cognitive and psychiatric symptoms, and women show faster cognitive decline after diagnosis of mild cognitive impairment or AD dementia. We hypothesized that sex differences in the expression of sex hormone receptors and Cytochrome P450 Family 19 Subfamily A Member 1 (CYP19A1), the aromatase enzyme that aromatize testosterone to estradiol, in the hippocampus (Hip) and anterior cingulate cortex (ACC) might be more pronounced between the subpopulation of AD subjects with MDD compared to AD. We examined the differences in transcript levels of estrogen receptor 1 (*ESR1*), *ESR2*, Progestin And AdipoQ Receptor Family Member 7 (*PAQR7*), also named as membrane progesterone receptor alpha, (*mPRα), PAGR8* (*mPRβ*) and *CYP19A1* in post-mortem brain samples from individuals with AD dementia and neuropathologic changes of AD, with (n=15) and without (n=19) prior depression. Hippocampal and ACC tissues were received from the Brain Bank of the Northwestern Cognitive Neurology and Alzheimer Disease Center. Transcript levels were determined by quantitative RT-PCR. Significant comorbidity differences were found in both Hip and ACC of AD+MDD samples compared to those of AD without MDD; *mPRα*, and *mPRβ* expressions were significantly lower in AD+MDD samples.

Expression of *CYP19A1* in the ACC was significantly and dramatically reduced in AD+MDD in both sexes, compared to that of AD without MDD. Expression of both *ESR1* and *ESR2* were significantly higher in the ACC of females with AD without MDD, but reduced in AD+MDD females and all males. These data suggest that sex hormone receptor regulation, and the availability of estradiol in the brain, could be altered by MDD in AD, and that this can lead to identifying novel drug targets in a subgroup of AD patients.

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Poster

P199. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches VIII

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P199.05

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

Support: NIA Grant AG057895

Title: Metabolomic analysis of mouse forebrain reveals that exercise may reverse some effects induced by loss of ovarian function and by an Alzheimer’s disease genotype

Authors: *J. WILLIAMS[1], E. WEN[1], E. A. FINCH[2], C. A. COLTON[3], J. W. THOMPSON[4], K. J. ADAMS[4], C. L. WILLIAMS[1];
[1]Dept. of Psychology & Neurosci., Duke Unviversity, Durham, NC; [2]RLA, Ctr. for Scientific Res., Rockland, MD; [3]Dept. of Neurol., Duke Univ. Med. Ctr., Durham, NC; [4]Duke Proteomics and Metabolomics Core Facility, Duke Univ. Sch. of Med., Durham, NC

Abstract: Alzheimer’s Disease (AD) disproportionately impacts women and the loss of ovarian hormones during perimenopause has been identified as a sex-specific risk factor. Physical exercise has neuroprotective effects for people with cognitive impairment and may be an effective therapeutic intervention for AD. While understanding of AD is limited by lack of biomarkers for diagnosis and treatment, metabolomics is a powerful tool to identify changes in biochemical pathways associated with disease progression and treatment. We sought to identify changes in cognition and forebrain metabolite levels in 36-week-old, CVN-AD mice (APPSwDI/mNos2$^{-/-}$) induced by a gradual loss of ovarian function (mouse model of transitional menopause) and by chronic aerobic exercise. Local immune suppression and arginine deprivation drive AD pathology in CVN-AD mice; by 36 weeks of age sedentary CVN-AD mice normally show memory loss and typical AD neuropathology. In this study, CVN-AD mice and control C57BL/6 and mNos2$^{-/-}$ mice were treated with either oil or 4-vinylcyclohexene diepoxide (VCD) to induce gradual ovarian failure. Half of the CVN-AD mice remained sedentary while the other half were exercised with both voluntary wheel running (access 5 days/wk) and forced

treadmill training (2 days/wk) for 12 wks beginning at 24 WOA, which is after cycling had ceased in the VCD group. All control mice remained sedentary. Novel object recognition tests at 36 WOA revealed that exercise improved short-term memory of CVN-AD mice, especially in mice that were no longer cycling. Following behavioral testing, mice were sacrificed and forebrain homogenates analyzed using the Biocrates MxP Quant 500 kit. As expected, arginine levels were decreased in CVN-AD mice compared to controls. However, exercise prevented this decrease, supporting its neuroprotective effects. Five metabolic pathways diverged between CVN-AD and C57 mice including aminoacyl-tRNA biosynthesis; glycine, serine, and threonine metabolism; arginine biosynthesis; alanine, aspartate, and glutamate metabolism; and glyoxylate and dicarboxylate metabolism. There was little overlap in the metabolites affected by ovarian failure across C57, NOS, and CVN-AD mice, suggesting that transitional menopause has differential effects in the 3 genotypes. Ceramides and sphingolipids increased significantly with exercise, though this effect was less pronounced in VCD mice. Overall, these results suggest that loss of ovarian function has differential effects on brain metabolic profiles in AD and non-AD mice and that exercise training can prevent some of the metabolic changes induced by an AD genotype and transitional menopause.

Disclosures: J. Williams: None. **E. Wen:** None. **E.A. Finch:** None. **C.A. Colton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent on CVN mouse model. **J.W. Thompson:** None. **K.J. Adams:** None. **C.L. Williams:** None.

Poster

P199. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches VIII

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P199.06

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

Support: AG058644
NIRG-04-1010

Title: Aerobic exercise alters the lipidome in mild cognitive impairment

Authors: T. J. GROSS[1], R. MALHAS[1], M. MONTGOMERY[2], F. MACCIARDI[1], A. K. CHEEMA[3], M. ESPELAND[4], S. O'BRYANT[5], C. W. COTMAN[1], L. BAKER[4], *M. MAPSTONE[1];
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Abstract: There is some controversy whether aerobic exercise benefits episodic memory in people with Mild Cognitive Impairment (MCI). The underlying mechanism of putative benefits of aerobic exercise may include alterations to lipid metabolism. The metabolism of polyunsaturated lipids including docosahexaenoic (DHA) and arachidonic (AA) polyunsaturated fatty acids (PUFAs) may impact the pathobiology of early Alzheimer's disease (AD). This disease-associated lipid dyshomeostasis may be improved with a sustained program of aerobic training. In a retrospective analysis of the Piedmont Aging, Cognition, and Exercise (PACE) study we considered 28 participants with MCI (> 50 yrs, 50% female, 18% APOE ε4 carriers) randomly assigned to Aerobic Exercise (n = 18) or Stretching (n = 10) arms. Prior to and following the 24-week study, participants contributed venous peripheral blood plasma specimens. We analyzed blood lipids using the 5500 QTRAP® LC-MS/MS (Sciex) targeted mass spectrometry platform. We modelled changes in PUFA metabolite abundances pre- and post-study as a function of intervention arm using restricted maximum likelihood (REML) regression as evaluated for each lipid. We found that tri-and di-acylglycerols (TAGs and DAGs) comprised the majority of altered DHA and AA PUFA-bearing lipids (89%), of which eight unique glycero-PUFA metabolites were altered (nominal $p < .05$) following active aerobic intervention. Subsequent analyses demonstrated that 33% of TAGs (but not DAGs) which incorporated any fatty acid in the DHA or AA biosynthetic pathway significantly varied due to aerobic exercise (five unique metabolites, nominal $p < .05$). Fatty acyl-glycerol dyshomeostasis has been frequently associated with aging-associated metabolic diseases. In addition, lipid dysmetabolism plays an increasingly studied and important role in early AD. These peripheral dynamics involving PUFAs support the notion of a physiologically extended brain-peripheral metabolic axis that is responsive to exercise intervention. Aerobic exercise may be useful for modifying the pathophysiological trajectory of AD, especially early in the course of the disease.

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Poster

P199. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches VIII

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P199.07

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

Support: AG067598
AG072599
AG061869

Title: Epichaperomics: a platform to decipher stressor-to-phenotype interactome alterations in alzheimer's disease

Authors: *S. D. GINSBERG[1,2,3,4], T. A. NEUBERT[5], H. ERDJUMENT-BROMAGE[5], A. RODINA[6], S. JOSHI[6], S. SHARMA[6], C. S. DIGWAL[6], T. WANG[6], G. CHIOSIS[6,7];
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Abstract: The recognition of the importance of complicated stressor-to-phenotype associations in human disease demands a greater understanding of how specific stressors affect interactomes, especially in the context of late onset neurodegenerative disorders such as Alzheimer's disease (AD). Currently untreatable diseases arise due to variations in, and through a combination of, multiple stressors of genetic, epigenetic, and environmental nature. Unfortunately, how these stressors lead to a specific disease phenotype or inflict a vulnerability to some cells and tissues but not others, remains largely unknown and unsatisfactorily addressed. This concept 'selective vulnerability' is especially relevant in AD and related neuropathological conditions, where specific neuronal populations and circuits degenerate while others do not, making treatment a vexing problem. Accordingly, analysis of cell- and tissue-specific interactome networks is posited to shed light on organization of biological systems and subsequently to disease vulnerabilities. To date, deriving human interactomes across different cell- and disease-contexts remains a challenge. We provide evidence linking stressor-induced protein interactome network perturbations to the formation of pathologic scaffolds termed epichaperomes. A specialized 'omics platform, epichaperomics is a viable and reproducible experimental solution to obtaining rigorous context-dependent interactomes. Epichaperomics can be performed as a standalone approach as well as complement and enhance currently available conventional approaches to aid the scientific community in defining, understanding, and ultimately controlling interactome networks of complex diseases such as AD. Ultimately, this approach may aid the transition from a limited single-alteration perspective in disease to a comprehensive network-based mindset, which we posit will provide an evidence based view how these maladaptive changes from stressor to phenotype provide unique precision medicine opportunities for diagnostic and therapeutic development, especially in the context of neurodegenerative disorders such as AD where treatment options are currently limited.

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Poster

P199. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches VIII

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P199.08

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

Support: BrightFocus ADF Fellowship

Title: Assessing genetic contributions to cerebrovascular damage in a novel MTHFR^{C677T} mouse model

Authors: *A. M. REAGAN[1], K. E. CHRISTENSEN[3], A. HABER[2], R. ROZEN[3], J. A. MEYER[4], J. PETERS[4], L. L. FIGUEIREDO[4], S. C. PERSOHN[4], A. A. BEDWELL[4], K. ELDRIDGE[4], R. SPEEDY[4], G. W. CARTER[1], M. SASNER[1], P. R. TERRITO[4], G. R. HOWELL[1];
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Abstract: Background: Mechanisms driving cerebrovascular decline during aging and in disease are poorly understood. Methylenetetrahydrofolate reductase (*MTHFR*) is a critical enzyme in the folate/methionine/homocysteine pathway. Variants in *MTHFR*, notably *C677T*, have been associated with AD, vascular dementia, and cardiovascular disorders. Approximately 30% of individuals carry at least one copy of *MTHFR^{C677T}*, causing a 50% decrease in MTHFR enzyme efficiency. Reduced efficiency can lead to high levels of homocysteine in blood, resulting in vascular inflammation and increased risk for cerebrovascular damage. A clinical trial aimed at reducing elevated homocysteine levels using dietary supplements did not slow cognitive decline in AD patients. We hypothesize that brain-specific vascular expression of *MTHFR^{C677T}* in cerebrovasculature drives damaging effects. **Methods**: CRISPR technology was employed to engineer the *Mthfr^{C677T}* allele into C57BL/6J (B6) mice. Vascular health is currently being assessed in 6, 18 and 24 months-old mice using immunofluorescence to examine blood brain barrier integrity in the cerebrovascular unit: endothelium (CD31) smooth muscle (αSMA) pericytes (PDGRFβ) basement membrane (ColIV) and tight junctions (Claudin-5, Occludin, ZO-1) as well as determining the presence of extravascular fibrin. Electron microscopy (EM) is used to examine cross sectional cerebrovascular ultrastructure. *In vivo* PET/CT imaging is utilized to assess blood flow. Bulk RNA sequencing and Reduced Representation Bisulfite Sequencing (RRBS) were performed on half brains to assess differential gene expression and DNA methylation, respectively. **Results**: Our preliminary data show that *Mthfr^{C677T}* mice phenocopy the human condition — mice show a 50% reduction in MTHFR enzyme activity in the liver and elevated levels of plasma homocysteine. Interestingly, young *Mthfr^{C677T}* mice also show a 50% reduction in MTHFR enzyme activity in the brain. PET/CT demonstrates reduced blood perfusion in female mice carrying the variant. In all mice carrying the variant, immunofluorescence shows reduced vascular density in the cortex, and EM reveals endothelial and pericyte apoptosis, reduced luminal size, and increased astrocyte and microglial presence in the microenvironment. **Conclusion**: We have created a novel mouse strain to examine mechanisms by which *MTHFR^{C677T}* could increase risk for vascular dysfunction. This work aims

to identify novel therapeutic approaches that reduce cerebrovascular pathology in AD and other dementias.

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Poster

P367. Spinal Cord Injury: Recovery II

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P367.01

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NJ Commission on Spinal Cord Research - CSCR14ERG007
NJ Commission on Spinal Cord Research - CSCR20ERG013

Title: Framework and Simulations for Quasi Real-time Access to Artifact-free Muscle Response during Neuromuscular Electrical Stimulation

Authors: *R. PILKAR[1], K. MOMENI[2], M. RAVI[2], E. GARBARINI[2], G. F. FORREST[3]; [1]Ctr. for Mobility and Rehabil. Engin. Res., [2]Reynolds Ctr. for Spinal Stimulation, [3]Kessler Fndn., West Orange, NJ

Abstract: Neuromuscular electrical stimulation (NMES) remains one of the effective rehabilitation modalities for addressing the recovery of neuromuscular function post spinal cord injury (SCI). NMES operates on a basic principle that the application of electrical current to a muscle nerve or muscle belly can elicit action potentials and cause muscle contraction in paralyzed muscles. It has been reported that the NMES interventions that combine volitional efforts are potentially more effective as combining patient's voluntary contractions with electrical stimulation (ES) produces the strongest contraction, targets neuroplasticity, and elicits activation of muscles and multiple modes of afferent stimulation. However, these interventions are limited by the fact that active monitoring of voluntary neuromuscular efforts via electromyography (EMG), particularly at the stimulated muscle, is unattainable due to the presence of stimulation artifact. Although recent developments have produced several software-based approaches for offline processing of EMG data to eliminate ES artifact, currently, no tool exists which will provide real-time access to the neuromuscular responses during NMES. In the current investigation, we evaluate the applicability of a novel, inverse fast Fourier Transform (iFFT)-based method for artifact removal in real-time and compare its performance with our previously published and validated empirical mode decomposition (EMD)-notch filtering method. For our simulations, we utilize pre-recorded EMG data from the rectus femoris (RF)

muscle during NMES (35 Hz, 300 μsec) from five able-bodied (AB) and five individuals with incomplete SCI. To simulate a real-time scenario, samples within the sliding window of N seconds (N = 0.5, 1, 1.5, and 2 seconds) of the raw RF EMG signal are sequentially fed to the iFFT algorithm, and artifact removal operation is performed. The entire raw EMG signal is simultaneously processed using the EMD-notch filtering method. The normalized root-mean-squared-error (nRMSE) between the two outputs are computed. The secondary validation is performed using the cross-correlation of iFFT processed output with the concurrent torque profiles. Based on the nRMSE and cross-correlation coefficients (r) for each window size (N), the window size with the lowest nRMSE and highest r will be used for future experimental validations. This investigation is a significant step towards developing a unique system that will potentially provide real-time access to the inherent neuromuscular responses (intensity of induced contraction, muscle fatigue, motor unit firing rates, etc.) during NMES.

Disclosures: R. Pilkar: None. **K. Momeni:** None. **M. Ravi:** None. **E. Garbarini:** None. **G.F. Forrest:** None.

Poster

P367. Spinal Cord Injury: Recovery II

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P367.02

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Tim Reynolds Foundation

Title: Tolerability and effects of a therapeutic suprathreshold high-frequency rTMS protocol in able-bodied individuals

Authors: *N. BRIHMAT[1], A. HOXHA[2], D. ALLEXANDRE[2], S. H. SALEH[2], J. ZHONG[3], G. H. YUE[4], G. F. FORREST[1];
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Abstract: High-frequency repetitive transcranial stimulation (HF-rTMS) is known to induce corticospinal excitability (CSE) increase and to have beneficial effect on motor function in individuals with motor deficits such after Spinal Cord Injury (SCI). A recent animal study has shown the beneficial regenerative and motor effects of a high-intensity, high-frequency rTMS protocol in a mice model of SCI. Here, we examine if a single session of a HF-rTMS protocol, equivalent to the one effective in mice, can be safely administered to able-bodied (AB) individuals. Four right-handed men (30.8 ± 1.9 years old) were included in this pilot study. Using a MagPro X100 device equipped with a figure-of-eight coil, HF-rTMS was delivered over

the left primary motor cortex, in 10 trains of 75 biphasic stimuli at a frequency of 15 Hz at 120% of the resting motor threshold (RMT). TMS safety and side-effects questionnaires were administered at the end of the session. The numeric rating scale (NRS) and Visual Analogue Scale (VAS) were administered to investigate any changes in pain. We assessed CSE with measures of resting (rMEP) and active (aMEP, at 10-25% maximal voluntary contraction) Motor Evoked Potential of the first dorsal interossei (FDI) muscle at 120% motor threshold and motor function using grip strength measures and the Box and Block test (BBT). The session was safely administered and well-tolerated by all the participants without any reported side-effect or pain development. Preliminary results reveals that a single session of HF-rTMS applied at 120% RMT induced an increase of CSE at rest (+24.8 ± 11.7% compared to baseline, d=1.12 using paired t-test) but not during active muscle contraction (-17.9 ± 28.8% compared to baseline, d=0.47). No significant changes were observed for grip strength and BBT measures. The results of this pilot study demonstrate that a single-session of HF-rTMS at 120%RMT can be safely administered to AB individuals without any adverse event. Ten minutes rTMS session induced CSE modulation suggesting that if repeated and combined with motor training could result in motor function improvements. These results should be considered when dealing with clinical population.

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Poster

P367. Spinal Cord Injury: Recovery II

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Program #/Poster #: P367.03

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIDILRR under Grant RERC 90RE5021-01-00
ARRT 90ARHF0002
Tim Reynolds Foundation

Title: Spinal cord transcutaneous stimulation combined with activity-based training can improve sitting balance in people with tetraplegia

Authors: *F. ZHANG, K. MOMENI, M. RAVI, A. BHEEMREDDY, R. PILKAR, G. F. FORREST;
Kessler Fndn., West Orange, NJ

Abstract: Cervical spinal cord injury (SCI) often leads to impairment or loss of motor and/or sensory function in upper limbs, trunk, and lower limbs, resulting in impaired sitting balance. Trunk balance is ranked as the third priority to improving the quality of life of people living with

tetraplegia, even more important than bladder/bowel function and walking. Non-invasive spinal cord transcutaneous stimulation (scTS) is a novel electrical neuro-modulation strategy that has the potential to increase the excitability of these spinal circuits and facilitate functional recovery after SCI. Our preliminary data have shown that low-intensity cervical scTS combined with short-term activity-based training (ABT) can lead to immediate and long-lasting (even further increased at 3 months follow-up) upper extremity (UE) functional recovery in people with complete and incomplete tetraplegia. The goal of this study is to investigate whether the scTS+ABT intervention can also improve sitting balance in individuals with tetraplegia. Three individuals with complete (ASIA Impairment Scale, AIS A) and incomplete tetraplegia (AIS B and D) were trained and tested this study. Each participant received a short-term intervention of scTS combined with ABT for 60 minutes/session and 2-3 sessions/week. Sub-threshold, tonic stimulation (monophasic, 1ms rectangular pulses, 5kHz carrier frequency) was delivered at cervical and thoracic spinal segments over the dorsal skin, when participants received ABT focusing on UE functional tasks. The results showed that scTS combined with ABT led to improved sitting balance in all three participants, even when no specific trunk training was provided. The sitting balance scales in Activity-based Balance Level Evaluation (ABLE) were dramatically increased at post-intervention in two individuals (gains: 6pts and 9pts). In the Modified Functional Reach Test (MRFT), all tested participants demonstrated improved forward reaching distances (increased by 252.78%, 255.56%, and 19.48%, respectively). More importantly, these gains in sitting balance maintained or even further improved during the 1-month follow-up period without receiving any intervention, suggesting the potential for long-term restoration of motor control. The research findings will provide preliminary data for a future large clinical trial to establish clinical efficacy and scientific evidence for understanding the underlying mechanism for functional and neurological recovery.

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Poster

P367. Spinal Cord Injury: Recovery II

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P367.04

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Tim Reynolds Foundation

Title: Improved gait kinematics with spinal cord transcutaneous stimulation during overground walking

Authors: *M. ANJARIA[1], K. MOMENI[1], M. RAVI[1], A. BHEEMREDDY[1], F. ZHANG[1], R. PILKAR[2], G. F. FORREST[1];

[1]Reynold Ctr. for Spinal Stimulation, West Orange, NJ; [2]Ctr. for Mobility and Rehabil. Engin. Res., Kessler Fndn., West Orange, NJ

Abstract: A previous study by our group has shown that spinal cord transcutaneous stimulation (scTS) improves gait kinematics in subjects with complete or incomplete spinal cord injury (SCI) in overground walking using individualized and optimal parameters of scTS such as frequency and amplitude. The goal of this study was to evaluate spatial-temporal parameters and joint kinematics for one participant with an incomplete spinal cord injury during one session of activity-based training with and without stimulation walking overground. Multiple cohorts of scTS was delivered with electrodes on spinal sites between thoracic and sacral spinal sites (cathodes) and bilateral iliac crests (anodes). The stimulation waveform consisted of a monophasic rectangular pulse with 1ms pulse width and 10kHz carrier frequency. Full-body kinematics (*Motion Analysis, Santa Rosa, CA)* were determined while participant walked with and without stimulation. Results show a significant effect of stimulation on intra and inter-limb hip and knee joint kinematics. When overground gait training with stimulation was compared to overground gait training without stimulation: i) the coefficient of variation for left hip and knee joint kinematics during continued stepping decreased by 31% and 35% respectively; ii) the coefficient of variation for right hip and knee joint kinematics during continued stepping decreased by 2.5% and 6.5% respectively and iii) the standard deviation for left and right leg stride length decreased by 81% and 41% respectively. Our preliminary results show that the usage of optimal and individualized scTS parameters during overground gait training can facilitate more consistent gait kinematics in participants with SCI.

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Poster

P367. Spinal Cord Injury: Recovery II

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P367.05

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Tim Reynolds Foundation

Title: Decreased Variability in Gait Kinematics While Walking in Exoskeleton with Spinal Cord Transcutaneous Stimulation

Authors: *A. BHEEMREDDY[1], K. MOMENI[1], M. RAVI[1], M. ANJARIA[1], F. ZHANG[1], R. PILKAR[2], G. F. FORREST[1];
[1]Reynolds Ctr. for Spinal Stimulation, [2]Human Performance and Engin. Res., Kessler Fndn., West Orange, NJ

Abstract: The goal of this study was to evaluate the efficacy of non-invasive spinal cord transcutaneous stimulation (scTS) on improvement of exoskeleton gait for motor incomplete spinal cord injury (SCI). ScTS was applied below motor threshold during exoskeleton training. Transcutaneous stimulation (scTS) was delivered on thoracic spinal sites (as cathodes) using individualized parameters of stimulation for an individual with a motor incomplete SCI. A series of temporal and spatial neurophysiological mapping sessions determined the individualized stimulation parameters. During training, a standardized method for the 6-minute walk test (6MWT) was collected weekly with and without stimulation within exoskeleton during over-ground walking sessions. With the 6MWT, additional motion data were collected using inertial measurement unit (IMU) sensors (APDM Opals, USA) placed on the lower limb segments, and trunk (lumbar). Data from IMUs determined bilateral hip, knee and ankle 3-axis joint angles for both stimulation and no-stimulation conditions during exoskeleton gait. For repeated gait cycles, immediate onset of stimulation significantly decreased standard deviation for mean bilateral hip, knee and ankle joint angles. For left hip and bilateral knee the decrease in standard deviation was at least 40%. Longitudinal results show efficacy of training using scTS for one individual with a motor incomplete SCI to facilitate less variable gait profile for people with SCI walking in exoskeleton.

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Poster

P367. Spinal Cord Injury: Recovery II

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Program #/Poster #: P367.06

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Tim Reynolds Foundation

Title: Spinal Cord Transcutaneous Stimulation Elicits Volitional Contraction in an Individual with Motor Complete SCI

Authors: *K. MOMENI[1], M. RAVI[2], A. BHEEMREDDY[2], F. ZHANG[2], R. PILKAR[3], G. F. FORREST[2];
[2]Ctr. for Spinal Stimulation, [3]Ctr. for Mobility and Rehabil. Engin. Res., [1]Kessler Fndn., West Orange, NJ

Abstract: Non-invasive spinal cord transcutaneous stimulation (scTS) is applied to one or multiple spinal segments for neuromodulation, targeting spinal circuity. For individuals with spinal cord injury (SCI), recent studies have shown that spinal sensorimotor networks can be activated when electrical stimulation is delivered to the corresponding spinal segments. Our

preliminary findings have shown that applying scTS with optimized individualized parameters for each individual could target different neural networks after SCI. The goal of this pilot study is to identify the individualized scTS parameters for restoring volitional control during the voluntary initiation and execution of a specific motor task for an individual with a motor-complete SCI. An individual with a motor-complete SCI lesion was recruited to participate in the study. Sub-threshold, tonic scTS was delivered at thoracic and lumbar spinal segments over the dorsal skin, anodes placed on the anterior iliac crests. scTS waveforms were monophasic, rectangular 1ms pulses with a carrier-wave frequency set at 5 kHz. Systematic modulations for stimulation parameters with intensities at subthreshold determined voluntary initiation and extension of left knee in a recumbent, gravity-neutral position. Surface electromyography data were recorded to monitor lower extremity muscle activity. Using several stimulation sites or cohorts (i.e., thoracic and lumbar segments) resulted in initiating and successfully executing targeted motor tasks. These preliminary findings highlight how scTS neuromodulation of the spinal circuitry can restore significant motor function for individuals with motor-complete SCI.

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Poster

P367. Spinal Cord Injury: Recovery II

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Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P367.07

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Tim Reynolds Foundation

Title: Identifying Optimal Spinal Levels and Spinal Cord Transcutaneous Stimulation Parameters Using EMG Responses after SCI

Authors: *M. RAVI[1], K. MOMENI[1], F. ZHANG[1], A. BHEEMREDDY[1], M. ANJARIA[1], R. PILKAR[2], G. F. FORREST[1];
[1]Reynolds Ctr. for Spinal Stimulation, [2]Ctr. for Mobility and Rehabil. Engin. Res., Kessler Fndn., West Orange, NJ

Abstract: Increasing number of researchers have shown that modulating the circuitry of the spinal cord by using non-invasive Spinal Cord Transcutaneous Stimulation (scTS), usually in combination with activity-based training, can enhance sensorimotor function in lower and upper limbs as well as help with mitigating spasticity for individuals with spinal cord injury (SCI). However, due to the non-invasive nature of scTS, it can be challenging to target specific motor pools for muscles or muscle groups necessary for specific tasks. Multi-site scTS stimulation using specific stimulation parameters (amplitude frequency and waveform mode/phase) and

electromyography (EMG) can determine optimal and individualized spatial and temporal physiological parameters of stimulation. We were able to recruit individuals with SCI (both complete and incomplete) and applied scTS at select spinal levels between cervical and coccyx using anodes placed on the anterior iliac crests bilaterally and EMG was recorded at key lower limb and abdominal muscles. Stimulation waveforms were either monophasic, biphasic or rectified. Carrier-wave frequency was set at 5 kHz for monophasic and biphasic modes of stimulation whereas there was no carrier frequency in the rectified mode. Systematic modulations of amplitude and frequency induced specific EMG responses for different spinal roots. The responses were analyzed by latency - Early Responses (<15ms after stimulation pulse) and Late Responses (>15ms). Results of the analyses, show that the stimulation induced EMG responses can be used to determine the individualized and optimal scTS parameters of subthreshold stimulation to target voluntary control of specific muscles after SCI.

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Poster

P376. Pain Processing: Itch: Models and Mechanisms II

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P376.01

Topic: D.02. Somatosensation

Support: NIH Grant NS087088
NIH Grant HL141269
Pfizer Aspire Dermatology Award to L.H.

Title: Distinct functional organization of itch-sensing neurons

Authors: *Y. XING, H. R. STEELE, H. HILLEY, T. NIEHOFF, R. NHO, K. LAWSON, L. HAN;
Sch. of Biol. Sci., Georgia Inst. of Technol., Atlanta, GA

Abstract: Diverse sensory neurons exhibit distinct neuronal morphologies with varieties of axon terminal arborizations subserving their functions. Due to its clinical significance, the subject of the molecular and cellular mechanisms of itch is undergoing intense study. However, a complete analysis of itch-sensing terminal arborization morphology is missing. Using a novel *MrgprC11*^{CreERT2} transgenic mouse line, we labeled a small subset of itch-sensing neurons that express multiple itch-related molecules including MrgprA3, MrgprC11, histamine receptor H1, IL-31 receptor, 5-HT receptor 1F, natriuretic precursor peptide B, and neuromedin B. By combining sparse genetic labeling and whole-mount PLAP histochemistry, we found that itch-sensing skin arbors exhibit free endings with extensive axonal branching in the superficial

epidermis and large receptive fields. We also found that itch-sensing neurons display distinct morphologies in their central arbors within the dorsal horn of the spinal cord when innervating different body locations. Our optogenetics study also showed that activating the nape of the neck and the hind paw can induce itch behaviors including scratching (nape of the neck) and biting (hind paw). Moreover, the hind paw showed an increased sensitivity to the external light input, suggesting regional differences in itch transmission. These results revealed the unique morphological and functional characteristics of itch-sensing neurons and provide novel insights into the basic mechanisms of itch transmission.

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Poster

P376. Pain Processing: Itch: Models and Mechanisms II

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Program #/Poster #: P376.02

Topic: D.02. Somatosensation

Support: NIH NS087088
NIH HL141269
Pfizer Aspire Dermatology Award

Title: Staphylococcus aureus virulence factors as novel mediators of itch

Authors: *Y. NHO[1], K. LAWSON[1], F. BANOVIC[2], L. HAN[1];
[1]Sch. of Biol. Sci., Georgia Inst. of Technol., Atlanta, GA; [2]Univ. of Georgia, Athens, GA

Abstract: Atopic dermatitis (AD) is one of the most common, debilitating chronic skin diseases that can often result in lifelong burdens for affected patients, without any available cures. *Staphylococcus aureus* (*S. aureus*) is a major human pathogen that has been found to have a significant presence on the lesional skin of AD patients. While there has been some work to investigate the relationship between *S. aureus* and pain, there has been little research focused on the contributions of the bacteria to itch, a major symptom of AD. Therefore, we examined *S. aureus* virulence factors to investigate cellular and behavioral mechanisms in which the bacteria contributes to AD. Calcium imaging of dorsal root ganglia (DRG) in $Pirt^{GCaMP3/+}$ mice revealed that 10μM of δ-Toxin activated an average of 5.17% of DRG, while 10μM of PSMα2 and PSMα3 activated 30.8% and 23.0%, respectively. These results suggest that *S. aureus* virulence factors can directly activate sensory neurons. To investigate *S. aureus*-mediated activation of specific itch sensing neuron populations, we looked at the overlap in calcium activation by itch-receptor agonists and virulence factors. Results showed overlap in calcium influx between the virulence factors and several itch agonists, including β-alanine, chloroquine, and sphingosine-1-

phosphate. These results indicate, for the first time, potential mechanisms for *S. aureus*-mediated itch through itch-sensing neurons populations including Mas-related g-protein coupled receptor (Mrgpr) D^+, $MrgprA3^+$, and natriuretic peptide B ($NPPB^+$). To examine the behavioral responses induced by individual *S. aureus* virulence factors, we used a cheek injection behavior test in C57BL/6, which effectively differentiates between itch and pain behavior through scratching and wiping, respectively. We found that δ-Toxin, PSMα2, and PSMα3 all induced significant scratching and wiping of the cheek. These results suggest that *S. aureus* virulence factors can mediate itch and pain behavior. In summary, our findings indicate new directions for future studies understanding the contributions of *S. aureus* in itch and AD, as well as the development of therapies against the bacteria in skin disease.

Disclosures: Y. Nho: None. **K. Lawson:** None. **F. Banovic:** None. **L. Han:** None.

Poster

P376. Pain Processing: Itch: Models and Mechanisms II

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Program #/Poster #: P376.03

Topic: D.02. Somatosensation

Support: NIH Grant NS087088
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Pfizer ASPIRE Dermatology

Title: Cerebellin family members are differentially expressed across sensory modalities

Authors: *C. HANSON, K. LAWSON, L. HAN;
Georgia Inst. of Technol., Atlanta, GA

Abstract: The cerebellins (Cbln) family is a group of secretagogues and trans-synaptic enhancers. These hexameric proteins form synapse spanning scaffolds with a pre-synaptic neurexin and post-synaptic delta ionotropic glutamate receptor (GRID). Although their role in synaptic organization has been probed throughout the brain, examination of cerebellins in the periphery and spinal cord has been sparse. We used RNAScope in situ hybridization to provide novel evidence of differential expression of cerebellin family members in distinct groups of dorsal root ganglia (DRG) neurons. Cbln1 is preferentially expressed by non-peptidergic C-fibers that co-express Mas-related G-protein coupled receptor A3 (MrgprA3); these neurons constitute a unique population specifically linked to itch. Cbln2 is expressed by two separate groups of A-fibers that both express mechanosensitive ion channel Piezo2. Two-thirds of Cbln2 neurons are medium sized and marked by Tropomyosin receptor kinase B (TrkB); this group likely represents Aδ low threshold mechanoreceptors (LTMRs). One-third of Cbln2 neurons are marked by parvalbumin (PV) and Tropomyosin receptor kinase C (TrkC) expression, suggesting

this group constitutes proprioceptive neurons. We additionally demonstrate that Cbln+ DRG neurons express an isoform of binding partner neurexin. Within the dorsal horn, Cbln1 expression is concentrated in lamina II, where resident interneurons receive nociceptive afferents. Cbln2 is expressed more ventrally, in regions that receive tactile input from LTMRs. These results support our hypothesis that Cbln family members are differentially expressed across sensory modalities, in both the central and peripheral nervous system.

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Poster

P376. Pain Processing: Itch: Models and Mechanisms II

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Program #/Poster #: P376.04

Topic: D.02. Somatosensation

Support: NIH Grant NS087088
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Pfizer Aspire Dermatology Award

Title: Mrgprc11+ drg sensory neurons mediate glabrous skin itch

Authors: *H. STEELE[1], Y. XING[2], Y. ZHU[1], H. HILLEY[1], K. LAWSON[1], Y. NHO[1], T. NIEHOFF[1], L. HAN[3];
[2]Biol. Sci., [3]Sch. of Biol. Sci., [1]Georgia Inst. of Technol., Atlanta, GA

Abstract: Although chronic itch is the most common reason for visiting the dermatologist, there remain few effective treatments. Additionally, glabrous skin (palms and soles) itch, has attracted little attention within the field due to a lack of methodology. This is despite glabrous skin itch, which arises from a variety of medical conditions such as plantar and palmar psoriasis, dyshidrosis, and cholestasis, being extremely debilitating. To identify the neuronal populations mediating glabrous skin itch we examined the central peripheral innervation pattern of three previously identified itch-sensing neurons (MrgprA3+, MrgprD+, and MrgprC11+). We found that both MrgprD+ and MrgprC11+ sensory neurons densely innervate glabrous skin, while MrgprA3+ sensory neurons do so only very sparsely. These results indicate, for the first time, the potential mechanistic differences that exist between hairy and glabrous skin itch. As MrgprC11+ neurons are previously uncharacterized, we used immunostaining and RNAscope in situ hybridization to determine that MrgprC11 is expressed in a subset of small-diameter peptidergic neurons that constitute ~18% of DRG sensory neurons, including the majority of both MrgprA3+ and NPPB+ itch-sensing neurons. To investigate and the mechanisms of glabrous skin itch, we developed novel acute and chronic mouse behavioral assays to examine itch sensation arising from the glabrous skin of the plantar hindpaw. We found that mice exhibit specific biting

behavior in response to pruritogens and licking in response to algogens. To confirm this specificity, we also used morphine as an analgesic. We found that although morphine significantly attenuated capsaicin-induced licking behavior, it had no effect on Bam8-22-induced biting behavior. Using this assay, we then demonstrated that chemogenetic activation of MrgprA3+ and MrgprD+ nerves in the glabrous skin do not induce biting behavior, suggesting that they do not mediate glabrous skin itch. In contrast, we found that activation of MrgprC11+ neurons using both chemogenetic activation and the MrgprC11 agonist Bam8-22 induced significant dose-dependent biting behaviors in glabrous skin. Finally, we found that ablation of MrgprC11+ neurons almost completely abolishes both acute (Bam8-22) and chronic glabrous skin itch. These results suggest that MrgprC11+ neurons are a major mediator of glabrous skin itch. In summary, our findings reveal new avenues for future glabrous skin itch study and the development of glabrous-skin specific anti-itch therapies.

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Poster

P376. Pain Processing: Itch: Models and Mechanisms II

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P376.05

Topic: D.03. Somatosensation – Pain

Title: The posterior insula contributes to both affective and sensory processing of pain and itch

Authors: *F. KADAKIA, S. DAVIDSON;
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Abstract: Pain and itch are distinct percepts but both are complex emotional, motivational, and cognitive somatosensory experiences that require supraspinal processing. The insular cortex is thought to be involved in encoding the affective and sensory components of nociceptive stimuli. However, it is unknown how the insula controls behavioral responses to nociceptive and pruriceptive stimuli. We hypothesized that the insula regulates the valence of somatosensory information and modulates sensory and affective pain and itch behavior. We conducted anatomical, physiological, and behavioral experiments to characterize the involvement of the insula in pain and itch processing. Anterograde and retrograde tracer experiments revealed that the posterior insula (PIC), not anterior, receives projections from the thalamic posterior triangular nucleus (PoT), a target for ascending somatosensory input. We then tested neural responses in the PIC to complete Freund's adjuvant (CFA)-induced inflammatory pain in the hindpaw which resulted in an increase in cFos-positive cells compared to naive, suggesting that the PIC processes nociception. Next we tested whether chemogenetic activation of excitatory neurons in the PIC altered aversion and somatosensory behavior. Virus expressing hM3D(Gq)

excitatory DREADDs was injected bilaterally into the PIC of naive mice. Activation of the PIC with DREADD agonist compound 21 produced a place aversion. Chemogenetic activation of the PIC also produced an increase in scratching in response to cheek injection of the pruritogen chloroquine. Finally, to determine how the PoT to PIC pathway influences nociceptive processing, we selectively activated the PoT to PIC projection with retro-cre/DIO DREADDs. This resulted in an increase in thermal threshold in naive mice. Together these results suggest the PIC activation promotes aversion or escape behavior to somatosensory stimuli while simultaneously raising thermal sensory threshold. We hypothesize that activation of a negative-emotional valance system in the PIC leads to descending inhibition of noxious somatosensation.

Disclosures: F. Kadakia: None. **S. Davidson:** None.

Poster

P376. Pain Processing: Itch: Models and Mechanisms II

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P376.06

Topic: D.02. Somatosensation

Title: Sustained anti-pruritic effect in mice with TP-2021, a kappa opioid agonist peptide, delivered by subdermal ProNeura Implants

Authors: B. B. LAND[1], S. SREEDHARAN[2], R. PATEL[2], T. BECK[3], K. DEVARNEY[2], M. RUBIN[2], *C. CHAVKIN[1];
[1]Pharmacol., Univ. of Washington, Seattle, WA; [2]Titan Pharmaceuticals, South San Francisco, CA; [3]Med. Univ. of South Carolina, Charleston, SC

Abstract: Chronic pruritus (itch) is associated with a number of serious medical disorders and results in severe, often debilitating itching without relief from conventional topical or systemic agents. Kappa opioid receptor (KOR) agonists have recently demonstrated their clinical potential for the treatment of moderate-to-severe chronic pruritus; however, activation of KOR in the CNS results in dysphoria and psychosis. TP-2021 (formerly called JT-09) is a D-amino acid tetrapeptide designed to relieve pruritus through peripheral KOR agonism therefore potentially mitigating these serious central adverse effects. TP-2021 has demonstrated high potency (EC_{50} = 52 pM) and selectivity for KORs with little to no affinity for mu or delta opioid receptors in the cAMP Hunter™ (Eurofins) assay with cloned human receptors. Pretreatment with TP-2021 or with difelikefalin, (Korsuva™ by Cara Therapeutics, Inc.), was observed to be equally potent in reducing scratching and grooming bouts when injected subcutaneously (0.3 mg/kg in saline) in a 5'-guanidinonaltrindole (5'-GNTI) itch-induced mouse model. Korsuva is under FDA review as a thrice-weekly IV injection in dialysis patients with chronic pruritus. Titan's clinically proven ProNeura® implantable technology is designed to treat chronic medical conditions, where maintaining consistent, around-the-clock blood levels of small molecule medications could

significantly improve patient outcomes. The first ProNeura-based product is a six-month buprenorphine implant, with marketing approvals in the US, Canada and Europe. First, TP-2021 ProNeura prototype implants were formulated and evaluated for release pharmacokinetics in mice compared to TP-2021 subcutaneous injections. Results indicate that a single implant provided sustained plasma concentrations of TP-2021 comparable with the levels observed after acute administration of TP-2021. Next, TP-2021 prototype implants were tested in the 5'-GNTI itch-induced mouse model. Following subdermal placement of one or two implants, the treated and untreated or placebo-implant treated mice were repeatedly challenged bi-weekly with 5'-GNTI. TP-2021 implants produced sustained, statistically significant itch suppression, compared to control implants, for two weeks before reverting to non-treated levels at four weeks. Further refinement of the ProNeura implant formulation is ongoing with the aim of developing a product that could potentially sustain therapeutic anti-pruritic levels of TP-2021 over several months. Disclosures: BBL & CC do not receive financial support from Titan Pharmaceuticals; TB is a consultant and SS, RP, KD & MR are employees of Titan.

Disclosures: B.B. Land: None. **S. Sreedharan:** A. Employment/Salary (full or part-time):; Titan Pharmaceuticals. **R. Patel:** A. Employment/Salary (full or part-time):; Titan Pharmaceuticals. **T. Beck:** F. Consulting Fees (e.g., advisory boards); Titan Pharmaceuticals. **K. DeVarney:** A. Employment/Salary (full or part-time):; Titan Pharmaceuticals. **M. Rubin:** A. Employment/Salary (full or part-time):; Titan Pharmaceuticals. **C. Chavkin:** None.

Poster

P376. Pain Processing: Itch: Models and Mechanisms II

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P376.07

Topic: D.03. Somatosensation – Pain

Support: NIH Grant R01NS086859
NIH Grant R21EY031849
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Title: The irritant receptor TRPA1 mediates the mosquito repellent effect of catnip

Authors: *M. CAPEK[1], N. MELO[2], O. M. ARENAS[1], A. AFIFY[3], A. YILMAZ[2], C. J. POTTER[3], P. J. LAMINETTE[1], A. PARA[1], M. GALLIO[1], M. C. STENSMYR[2]; [1]Dept. of Neurobio., Northwestern Univ., Evanston, IL; [2]Dept. of Biol., Lund Univ., Lund, Sweden; [3]Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Catnip (*Nepeta cataria*) is a common garden herb well known for its euphoric and hallucinogenic effects on domestic cats, for its medicinal properties, as well as for its powerful repellent action on insects. Catnip extracts have been proposed as a natural alternative to synthetic insect repellents, such as N,N-diethyl-3-methylbenzamide (DEET), but how catnip triggers aversion in insects is not known. Here, we show that, both in *Drosophila melanogaster* flies and *Aedes aegypti* mosquitoes, the major mediator of catnip repellency is the widely conserved chemical irritant receptor TRPA1. *In vitro*, both catnip extract and its active ingredient nepetalactone can directly activate fly and mosquito TRPA1. *In vivo*, *D. melanogaster* and *Ae. aegypti* TRPA1 mutants are no longer repelled by catnip and nepetalactone. Interestingly, our data show that some, but not all, fly and mosquito TRPA1 variants are catnip targets. Moreover, unlike the broad TRPA1 agonist allyl isothiocyanate (AITC) (an active ingredient of tear gas and wasabi), catnip does not activate human TRPA1. Our results support the use of catnip and nepetalactone as insect-selective irritants and suggest that, despite TRPA1's broad conservation, insect TRPA1 can be targeted for the development of safe repellents.

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Poster

P397. Peripheral Mechanisms of Neuropathic Pain

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P397.01

Topic: D.03. Somatosensation – Pain

Support: Saint Louis University NeuroSURF Undergraduate Student Grant
R01NS113257-01S1

Title: Optimization of the HiBit peptide to understand the GPR160 and CARTp binding mechanism

Authors: *L. SARVA[1], Y. KYRIUKHA[2], D. SALVEMINI[3], J. CHRIVIA[4], S. KOROLEV[2]; [2]Biochem. and Mol. Biol., [3]Pharmacol. and Physiological Sci., [1]St. Louis Univ., St. Louis, MO; [4]Pharmacol. and Physiological Sci., St. Louis Univ. Sch. of Med., St. Louis, MO

Abstract: Opioids have long been used to treat neuropathic and chronic pain; however, their addictive nature has resulted in extreme misuse. The ongoing opioid crisis and the hesitation to treat with these drugs have deemed it necessary to find alternative treatment options. G protein-coupled receptors are promising areas to explore. Specifically, GPR160, a newly de-orphaned receptor protein has been shown to directly relate to the inhibition of pain perception and metabolic activity when it is bound to its ligand CARTp. GPR160 has not been isolated yet,

therefore studying its interaction is incredibly difficult. To study the CARTp interaction with GPR160 we employed a nano luciferase-based complementation bioluminescence assay. CARTp is fused to a HiBit peptide and GPR160 is tagged with an LgBit luciferase. When the HiBit peptide activates LgBit, it triggers a secondary interaction between the activated LgBit and the substrate. This produces luminescence indicating that HiBit has bound to LgBit. In order to use this as a readout between CARTp and GPR160, the affinity of the HiBit peptide to LgBit must be optimized. Thus, the CARTp peptide is expressed in E. coli and purified using affinity chromatography steps. GPR160 is expressed in THP-1 cells. We introduced various mutations of HiBit to develop variant suitable for this purpose. The implications of this confirmatory project extend to understanding the breadth of the CARTp and GPR160 interaction and its function. It also extends to increased understandings of membrane protein purification and their correlation to pain.

Disclosures: L. Sarva: A. Employment/Salary (full or part-time):; Saint Louis University. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Saint Louis University NeuroSURF Undergraduate Student Grant. **Y. Kyriukha:** None. **D. Salvemini:** None. **J. Chrivia:** None. **S. Korolev:** None.

Poster

P397. Peripheral Mechanisms of Neuropathic Pain

Location: Virtual Only

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Program #/Poster #: P397.02

Topic: D.03. Somatosensation – Pain

Support: NIH Grant T32 GM008600
NIH Grant P30 CA014236
R01AR071722
Duke University Anesthesiology Research funds
Duke Microbiome Center Grant

Title: Sting-mediated type-I interferon signaling suppresses osteoclastogenesis and bone fracture pain

Authors: *M. LEE, K. WANG, A. MCGINNNIS, R.-R. JI, C. R. DONNELLY;
Pain Res. Division, Anesthesiol., Duke Univ. Med. Ctr., Durham, NC

Abstract: STING, or stimulator of interferon genes, is an intracellular DNA sensor that serves a crucial role in innate immunity by promoting immune cell-mediated elimination of pathogens and damaged host cells via the induction of type-I interferons (IFN-I) and other cytokines. In addition to its critical functions in immunity and host defense, we recently demonstrated that the STING/IFN-I signaling axis is a potent regulator of pain (nociception). Interestingly, we found

that autocrine or paracrine activation of STING in sensory neurons leads to the production and release of IFN-I, which directly suppresses the excitability of nociceptors. Interestingly, IFN-I signaling has previously been shown to inhibit osteoclastogenesis in naïve mice, and mice deficient for IFN-β or the IFN-I receptor component Ifnar1 exhibit reduced bone density. Bone fracture is not only remarkably painful, but also leads to an extended period in which fracture healing is impaired by inflammation-induced osteoclast overactivation. Thus, in this study we sought to determine the effects of STING agonists on pain, locomotor function, and osteoclastogenesis using a murine model of bone fracture. Notably, we found that acute treatment with the STING agonists DMXAA and ADU-S100 could transiently attenuate behavioral signatures of fracture-induced bone pain. In contrast, repeated administration of STING agonists following bone fracture led to prolonged pain control and improved locomotor recovery. Based on these data, we hypothesized that STING agonists may exert their effects via direct actions on sensory neurons and via direct actions on osteoclasts. In support of this hypothesis, we found that STING agonists dose-dependently inhibited osteoclastogenesis *in vitro*, as measured by differentiation of bone marrow-derived macrophages (BMDMs) or RAW264.7 cells to osteoclasts. Importantly, these effects were dependent on IFN-I signaling, as STING failed to suppress osteoclastogenesis in BMDMs from *Ifnar1*$^{-/-}$ mice and in RAW264.7 cells in the presence of anti-IFN-I antibodies. Based on these data, we conclude that activation of the STING pathway may be of therapeutic utility in fracture-induced bone pain.

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Poster

P397. Peripheral Mechanisms of Neuropathic Pain

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Program #/Poster #: P397.03

Topic: D.03. Somatosensation – Pain

Support: NIH T32 GM008600
NIH P30 CA014236

Title: Expression of PD-1 in T cells in the peripheral nervous system after neuropathy and its possible role in neuropathic pain

Authors: *A. MCGINNIS[1], R.-R. JI[3], Z. WANG[1], J. ZHAO[4], O. CHEN[2];
[2]Cell Biol., [1]Duke Univ., Durham, NC; [3]Pain Res. Division, Anesthesiol., [4]Duke Univ. Med. Ctr., Durham, NC

Abstract: It is well appreciated that T cell populations affect neuropathic pain pathogenesis, but the molecular underpinnings of their effects are poorly understood compared to other immune cell types. Programmed cell death ligand-1 (PD-L1) is best know to be expressed by cancer cells and to suppress T cell-mediated immunity through activation of its receptor, PD-1, which is abundantly expressed by T cells. We recently demonstrated that PD-1 is also expressed by primary sensory neurons and inhibits synaptic transmission, pain, and opioid analgesia (Chen et al., 2017; Wang et al., 2020). We aim to utilize PD-1's inhibitory effect on T cells to selectively study the contribution of T cell subtypes to pain. In our initial experiments, we sought to: 1) examine PD-1 expression in T cells in the peripheral nervous system (PNS) after nerve injury; 2) characterize T cells infiltrating the PNS after injury (3) determine whether altered PD-1 signaling in T cells contributes to neuropathic pain after nerve injury. To this end, we generated PD-1 reporter mice and examined PD-1 expression in the sciatic nerve and dorsal root ganglion (DRG) of naïve mice and mice with partial sciatic nerve injury (PSNL, 1 week). Both tissues saw notably increased PD-1 levels after PSNL. Double staining with Iba1 showed most but not all PD-1 positive cells to be macrophages, with remaining 1ba1- cells bearing morphological features of T cells. Present studies are underway to characterize these PD-1+ cells using immunohistochemical and flow cytometry assays. We are also generating conditional knockout (cKO) mice by crossing *Pdcd1*$^{fl/fl}$ mice with *Foxp3*-cre mice to investigate the role of PD-1 in regulatory T cells.

References

Chen, G., Kim, Y. H., Li, H., Luo, H., Liu, D.-L., Zhang, Z.-J., . . . Ji, R.-R. (2017). PD-L1 inhibits acute and chronic pain by suppressing nociceptive neuron activity via PD-1. *Nature neuroscience, 20*(7), 917-926. Wang, Z., Jiang, C., He, Q., Matsuda, M., Han, Q., Wang, K., . . . Ji, R.-R. (2020). Anti-PD-1 treatment impairs opioid antinociception in rodents and nonhuman primates. *Science translational medicine, 12*(531).

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Poster

P397. Peripheral Mechanisms of Neuropathic Pain

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Program #/Poster #: P397.04

Topic: D.03. Somatosensation – Pain

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Research to Prevent Blindness Unrestricted Departmental Grant (UIC Opth & Vis Sci)

Title: Differential Effects of Two Ocular Surface Injury Models on Function and Structure of Regenerating Corneal Cold Sensitive Nerves

Authors: *E. IVAKHNITSKAIA[1], T. NGUYEN[1], V. H. GUAIQUIL[1], Q. ZHOU[1], J. SARKAR[1], H. HIRATA[1], K.-Y. TSENG[2], M. I. ROSENBLATT[1];
[1]Ophthalmology and Visual Sci., Univ. of Illinois - Chicago, Chicago, IL; [2]Anat. and Cell Biol. / Neurosci., Univ. of Illinois At Chicago - Col. of Med., Chicago, IL

Abstract: Corneal nerve dysfunction can manifest as variable sensory disturbances from the ocular surface, spanning from the complete absence of sensation to chronic dysesthesia or pain. Corneal cold sensitive nerves (CSN) are a major functional subtype of corneal innervation and can be subdivided further into two subclasses. Low-Threshold (LT)-CSN regulate basal tearing and blinking, while High Threshold (HT)-CSN are likely responsible for the sensation of pain as well as reflex tearing and blinking responses. The impact of different forms of ocular surface injury on corneal sensation is incompletely understood. This study tested the hypothesis that different corneal nerve injury models lead to discrete alterations in the activity profiles of corneal CSN. We used in vivo extracellular electrophysiological recordings from the trigeminal ganglion and corneal wholemount immunostaining for TUJ1 to compare the functional and structural consequences of CSN regeneration from two clinically relevant models of injury. The first model, Corneal Epithelial Debridement (CED), is a mechanical injury that removes the dense nerve endings that innervate the cornea and was induced using a rotary burr tool. The second model, use of Benzalkonium Chloride (BAK), is a chemical injury that consists of a prolonged (30min+) ocular instillation with 0.01% BAK, a common preservative found in eye drops that can damage corneal epithelium and nerves. Sprague Dawley rats of both sexes were evaluated at 2 and 4 weeks post injury. We found that compared to uninjured corneas (n=14), nerve density is significantly reduced 2 weeks after nerve injury with either CED (n=7) or BAK (n=9) and is restored at 4 weeks post injury in the CED (n=8) but not the BAK injured group (n=7). Since the functional profiles of CSN at 2 vs 4 weeks post either CED or BAK injury did not differ, results from the two time points were combined for further functional analysis and compared to uninjured controls (n=24 LT-CSN, n=11 HT-CSN). We found that following regrowth from CED injury HT-CSN are less sensitive to ocular dryness and hyperosmolar stress (n=12). Unlike the CED group, HT-CSN regenerating from BAK injury (n=8) displayed significantly increased responsiveness to heating of the ocular surface and acute temperature drops. In contrast, the functional profiles of Low Threshold (LT)-CSN did not appear to be altered after either injury (n=5 for CED, n=9 for BAK). Overall, these results suggest that different injury models have

distinct impacts on the properties of corneal nerves regenerating from damage and that the pain-evoking HT-CSN subtype may be preferentially altered during regeneration from injury.

Disclosures: E. Ivakhnitskaia: None. **T. Nguyen:** None. **V.H. Guaiquil:** None. **Q. Zhou:** None. **J. Sarkar:** None. **H. Hirata:** None. **K. Tseng:** None. **M.I. Rosenblatt:** None.

Poster

P397. Peripheral Mechanisms of Neuropathic Pain

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P397.05

Topic: D.03. Somatosensation – Pain

Support: Blaustein Pain Research Grant (Johns Hopkins)

Title: Skin reinnervation by collateral sprouting after peripheral nerve injury

Authors: *S.-M. JEON[1], A. PRADEEP[1], L. MCDONOUGH[1], A. LATREMOLIERE[1], L. K. CRAWFORD[2], M. J. CATERINA[1];
[1]The Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [2]Univ. of Wisconsin - Madison, Madison, WI

Abstract: Peripheral nerve injury can result in loss of cutaneous sensory function, with associated defects in protective reflexes and tactile function, as well as neuropathic pain. While regeneration from the injured nerve is the best-studied mechanism of reinnervation following injury, it is inherently slow even with surgical augmentation. Collateral sprouting from neighboring uninjured neurons represents an additional mechanism for recovery. In this study, we aim to define the subtype specificity of sprouting mediated skin reinnervation after peripheral nerve injury and then correlate this anatomical recovery with sensory function. We have performed whole-mount immunofluorescence staining of hind paw skin in the mouse spared nerve injury (SNI) model, in which the tibial and peroneal branches of the sciatic nerves are ligated and transected, leaving the sural nerve intact. 7 days after SNI, we observed a large reduction in immunoreactivity (IR) for peptidergic (CGRP) and myelinated (NF-H) nerve fibers in the middle of the ipsilateral hind paw skin. However, progressive reinnervation by CGRP and NF-H nerve fibers from medial and lateral territories into denervated skin was observed at 28 and 56 days after injury. To ask whether low threshold mechanoreceptor (LTMR) subtypes participate in collateral sprouting after nerve injury, we have performed SNI surgery on transgenic mice selectively labeling the LTMR subtypes. An obvious injury-induced reduction in nerve terminal structures was observed for Aβ RA-LTMRs, Aδ RA-LTMRs, Aβ SAI-LTMRs, Aβ Field-LTMRs, and C-LTMRs in the ipsilateral hind paw. Even 56 days after SNI, collateral sprouting of most LTMR fibers into the denervated region was not evident. However, interestingly C-LTMR fibers sprouted new collaterals into denervated skin by 56 days. To define

the functional recovery of collateral sprouts in denervated skin after nerve injury, we carried out optogenetic experiments in $Pirt^{Cre}$; $Rosa26^{LSL\text{-}ChR2\text{-}EYFP}$ mice. Light-induced paw withdrawal frequency on the ipsilateral hind paw of $Pirt^{Cre}$; $Rosa26^{LSL\text{-}ChR2\text{-}EYFP}$ mice dropped dramatically by 3 days after SNI, but returned gradually to basal level over 56 days. We also quantified mechanical sensitivity, utilizing calibrated von Frey monofilaments and an Austerlitz pin applied to the midline of the hind paw. The response frequency to both stimuli decreased markedly 3 days after SNI, but returned gradually to basal level by 28 days. Our findings provide a rationale for targeting specific sensory neuron populations as a therapeutic strategy to promote the progression of sensory recovery and limit unwanted events such as chronic pain after peripheral nerve injury.

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Poster

P397. Peripheral Mechanisms of Neuropathic Pain

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P397.06

Topic: D.03. Somatosensation – Pain

Support: NIH Grant R01NS103812

Title: Dorsal root ganglionic field stimulation takes analgesic effects by activating Ca^{2+}/calmodulin-dependent protein kinase II

Authors: *H. TRAN[1], D. CHAO[2], Q. H. HOGAN[1], C. M. MECCA[3], B. PAN[2]; [2]Anesthesiol., [1]Med. Col. of Wisconsin, Milwaukee, WI; [3]Anesthesiol., Med. Col. of Wisconsin, Wauwatosa, WI

Abstract: Dorsal root ganglion (DRG) field stimulation (GFS) is effective in relieving clinical pain associated with nerve injury and neuropathic pain in animal models. Its molecular mechanism of analgesic effects has not been determined. Examining molecular mechanism of analgesic effects of GFS will provide us an opportunity to find the key molecule in the development of neuropathic pain. In our previous report, we found that there is reduced Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) activity after painful nerve injury and reduced CaMKII activity plays an important role in hypersensitivity to touch. We hypothesize that GFS might take analgesic effects by activating CaMKII activity. Intra-DRG injection of CaMKII activity inhibitors, KN-93 and autocamtide-2-related inhibitory peptide (AIP), blocked analgesic effects of GFS in rats with tibial nerve injury (TNI). With western blot and immunohistochemistry, we found that GFS reversed reduced protein level of phosphorylated CaMKII (pCaMKII) in DRG neurons from rats with TNI. With teased dorsal root single unit

electrophysiological recordings, we found that intra-DRG injection of AIP blocked effects of GFS on action potential propagation from peripheral sites. Taken together, CaMKII activity controls the analgesic effects of GFS and CaMKII might be one of the key molecules in the development of neuropathic pain.

Disclosures: H. Tran: None. **D. Chao:** None. **Q.H. Hogan:** None. **C.M. Mecca:** None. **B. Pan:** None.

Poster

P397. Peripheral Mechanisms of Neuropathic Pain

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Program #/Poster #: P397.07

Topic: D.03. Somatosensation – Pain

Support: DFG Grant SFB1158/2- TP 01
DFG Grant SFB1158/2- TP 04

Title: Action potential firing increases NKCC1 activity to stabilise axonal excitability during ongoing firing in mouse C-fibres

Authors: *R. CARR[1], K. SOHNS[1], V. BONALUME[3], A. KOSTENKO[1], R. RUKWIED[1], V. MAGNAGHI[3], M. SCHMELZ[2];
[1]Exptl. Pain Res., [2]Heidelberg Univ., Mannheim, Germany; [3]Pharmacol. and Biomolecular Sci., Univ. of Milan, Milan, Italy

Abstract: Somatosensory neurons resident in dorsal root and trigeminal ganglia maintain elevated intracellular chloride levels whereby activation of $GABA_AR$ leads to depolarisation and pre-synaptic inhibition in afferent nerve terminals within the spinal dorsal horn. We report here that the peripheral axons of DRG neurons are also depolarised by GABA and that GABA increased axonal electrical excitability. Increases in axonal excitability were predicated on NKCC1 activity and were larger during higher rates of action potential firing. To avoid the confounding effect of ongoing electrical stimulation used to determine axonal excitability, calcium responses to GABA were monitored in DRG neurons using the intensity based indicator Fluo8. DRG neuronal calcium responses to GABA were mediated by $GABA_AR$ and responses diminished in amplitude with successive GABA applications. This reduction in GABA response upon repeat application was exacerbated by NKCC1 blockade but prevented if, in the intervening period, either potassium (45mM) or electrical stimulation (30s @ 20Hz) was applied. This further implicated elevated action potential activity in the loading of DRG neurons with chloride via NKCC1. The physiological implication of activity-dependent chloride regulation in C-fibre axons was explored using activity-dependent conduction velocity slowing (ADS) to quantify excitability loss during 2.5 Hz challenge. ADS was reduced by $GABA_AR$ agonists and

exacerbated by either $GABA_AR$ antagonists or NKCC1 blockade. This illustrates that activation of $GABA_AR$ stabilised C-fibre excitability during sustained firing. We posit that NKCC1 activity increases in proportion to action potential firing rate. This allows axons to maintain their elevated intra-axonal chloride concentration. The resulting chloride gradient can be utilised by $GABA_AR$ to stabilise axonal excitability during ongoing firing. The data imply that therapeutic strategies targeting axonal chloride regulation at peripheral loci of pain and itch may curtail aberrant firing in C-fibres.

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Poster

P397. Peripheral Mechanisms of Neuropathic Pain

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Program #/Poster #: P397.08

Topic: D.03. Somatosensation – Pain

Support: NIH Grant HD093858 (SM)
NIH Grant NS11892 (KB)

Title: Endometriosis (ENDO) induced vaginal hyperalgesia in the rat: establishing the relationship between endocannabinoids (eCBs) and related lipids during development.

Authors: E. ARTHURS[1], A. VARNADOE[2], L. LANG[1], M. VASU[3], ***S. MCALLISTER**[1]; [1]Emory Univ. Sch. of Med., Atlanta, GA; [2]Cornell Univ. Col. of Vet. Med., Ithaca, NY; [3]Univ. of California, Berkeley, Berkeley, CA

Abstract: Endometriosis is a painful disorder defined by the growth of endometrial tissue outside the uterus (cysts). How the cysts contribute to painful symptoms such as dyspareunia (vaginal hyperalgesia) is poorly understood. A rat model of endometriosis (ENDO) is created by autotransplanting pieces of uterus onto abdominal arteries (Vernon & Wilson, 85). These cysts are associated with painful symptoms including vaginal hyperalgesia similar to women with endometriosis. In both rats and women, the cysts recruit their own sensory and sympathetic nerve supply (Berkley et al., 04; 05). Previous studies suggest that the cyst sensory and sympathetic innervation contribute to the development and maintenance of endometriosis-associated vaginal hyperalgesia (McAllister et al, 09; 12; Zhang et al., 08). Further, the cyst sensory and sympathetic innervation express cannabinoid receptors (CB1) and CB1 receptor agonists decrease, whereas CB1 receptor antagonist increase, endometriosis-associated hyperalgesia (Dmitrieva et al, 10; McAllister & Sinharoy, 18). These findings, with the knowledge that the endocannabinoid system (ECS) is involved in uterine function and dysfunction and that exogenous cannabinoids have been used for centuries to alleviate dysmenorrhea, suggest that the

ECS is involved in both endometriosis and its associated pain (Dmitrieva & Berkley, 02; Bradshaw & Walker, 04; Russo, 02). To test this hypothesis further, we analyzed endogenous cannabinoid levels during the time period when cyst innervation and vaginal hyperalgesia are known to develop in the rat model of endometriosis (McAllister et al., 2012). Lipid extraction and tandem mass spectrometry were used to assess levels of eCBs (AEA, 2-AG, NAGly) and related lipids (PEA, OEA, DHEA, SEA) in the ectopic uterus (cysts) of ENDO rats and the eutopic uterus of ENDO rats and controls. All samples were analyzed in estrous stage of proestrus. Overall, results demonstrate that lipid levels are significantly lower in ENDO rats compared to controls. These data suggest that altered eCB biosynthesis and/or metabolism in endometriosis may influence endometriosis-induced vaginal hyperalgesia, perhaps by reduced cannabinoid receptor activation on cyst innervation. These findings provide further support for the involvement of the ECS in mechanisms underlying endometriosis and its associated pain, thereby providing a novel approach for the development of badly-needed new treatments.

Disclosures: E. Arthurs: None. **A. Varnadoe:** None. **L. Lang:** None. **M. Vasu:** None. **S. McAllister:** None.

Poster

P397. Peripheral Mechanisms of Neuropathic Pain

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P397.09

Topic: D.03. Somatosensation – Pain

Support: NSF CAREER – 1844762
NIH U01 NS 113873

Title: Use visceral motor responses to colorectal distension to assess invasive neuromodulatory protocols for managing visceral pain

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Abstract: Managing visceral pain remains an unmet clinical need and conventional pharmacological treatments often lead to severe side effects that outweigh the therapeutical benefits. Neuromodulation has emerged as a non-drug alternative with growing clinical success in relieving chronic pain in joints, muscles, and nerves. Small-scaled clinical studies have indicated neuromodulation as a promising means to treat visceral pain. New neuromodulatory protocols need to be developed and validated in animal models to allow a mechanistic understanding of the pain-relieving effect by neuromodulation. Visceral motor reflex (VMR) to graded colorectal distension (CRD: 15, 30, 45 and 60 mmHg pressure steps) has been extensively implemented to assess the level of visceral pain in awake rodents, which unavoidably

produce movement artifacts to confound the VMR recordings. A single urethane injection preserves the spino-bulbo-spinal reflex to allow the recording of VMR to CRD in anesthetized rats, but it appears to provide unreliable level of anesthesia in mice. Here, we report an optimized protocol with prolonged urethane infusion that allows robust and repeatable recordings of VMR to CRD in mice under deep anesthesia, providing a 3-hour window to assess invasive neuromodulatory protocols. C57BL/6 mice of both sexes (8-16 weeks, 25-40 g) were anesthetized by 2% isoflurane inhalation during all surgical procedures. A dose of 0.4g/kg urethane was administered intraperitoneally before surgery. Two pairs of teflon-coated stainless steel wire electrodes were sutured to the oblique abdominal musculature immediately above the inguinal ligament (both sides). A flexible plastic-film balloon (Φ8 mm x 15 mm) was inserted intra-anally to maintain the 5 mm distance between the end of the balloon and the anus by taping the connected inflating catheter to the tail. 30 minutes after the electrode placement and balloon insertion, another catheter was placed intraperitoneally beneath transversus abdominis musculature and urethane was administered continuously at 0.05~0.1 µg per kg per hour (µg/kg·h) via a syringe pump. This protocol allows mice under deep anesthesia without spontaneous or volitional movement for at least 3h in all 8 mice tested. VMR to CRD was reliability recorded from 8 mice for at least 3h. We applied this *in vivo* behavioral model to assess the effect of L6 DRG stimulation in suppressing visceral pain from distal colon and rectum. Stimulation of 50 and 100 Hz at L6 DRG reversibly blocked the VMR to CRD. This validated *in vivo* mouse model of prolonged urethane anesthesia will allow objective assessment of various invasive neuromodulatory strategies that alleviated visceral pain.

Disclosures: S. Zhang: None. **L. Chen:** None. **B. Feng:** None.

Poster

P397. Peripheral Mechanisms of Neuropathic Pain

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Topic: D.03. Somatosensation – Pain

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NIH Grant R01DA047089
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Title: The pathway of cMyc-EZH2-SIRT3 plays an important role in HIV-related neuropathic pain model

Authors: X. ZHU[1,2], H. YI[1], J. GU[1], K. HAYASHI[1], K. TAKAHASHI[1], Y. KASHIWAGI[1], S. LIU[1], H. LI[2], ***S. HAO**[1];

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Abstract: Chronic opioid application enhances HIV entry into immune cells. Emerging clinical data demonstrate that repeated use of opioid pain killers increases neuropathic pain in people living with HIV. Therefore, it is important to elucidate the molecular mechanisms of HIV-related chronic pain. HIV-1 envelope protein gp120 increases the expression of transcription factor c-Myc in cell proliferation. Morphine increases c-Myc expression. Epigenetic writer, EZH2 is a histone methyl transferase subunit of a polycomb repressor complex. EZH2 silences gene expression by generating a methylated epigenetic mark at H3K27me3. Sirtuins 3 (Sirt3) deacetylates MnSOD and thereby activates it for scavenging mitochondrial superoxide. In the present study, we investigated the pathway of cMyc, EZH2, and Sirt3 in gp120 with morphine (gp120/M)-induced neuropathic pain state using epigenetic approach. HIV-related neuropathic pain was induced by repeated administration of intrathecal recombinant gp120 with morphine. Protein expression of spinal cMyc, EZH2, and SIRT3 was examined using Western blots. Intrathecal antisense oligonucleotides against cMyc, GSK126 (EZH2 inhibitor) or recombinant Sirt3 (rSirt3) was given intrathecally. HIV-gp120/M upregulated the expression of cMyc and EZH2, and down-regulated Sirt3 in the spinal dorsal horn. Intrathecal administration of antisense oligonucleotides against cMyc, GSK126 (EZH2 inhibitor), or recombinant rSirt3 reduced mechanical allodynia and thermal hyperalgesia in the gp120/M pain model. Knockdown of cMyc reduced spinal EZH2 expression in gp120/M rats. Chromatin immunoprecipitation (ChIP) assay showed that **the enrichment of** cMyc binding to *ezh2* gene promoter region was increased in gp120/M treated rats compared to that in control group. Spinal GSK126 reversed the lowered Sirt3 expression in gp120/M rats. ChIP assay showed that **the enrichment of H3K27me3** binding to *sirt3* gene promoter region was increased in gp120/M treated rats compared to that in control group. These results demonstrated that spinal cMyc-EZH2-H3K27me3-Sirt3 pathway played an important role in the HIV gp120-related neuropathic pain state, and provided a new insight into novel therapy for HIV/opioid-related neuropathic pain.

Disclosures: X. Zhu: None. **H. Yi:** None. **J. Gu:** None. **K. Hayashi:** None. **K. Takahashi:** None. **Y. Kashiwagi:** None. **S. Liu:** None. **H. Li:** None. **S. Hao:** None.

Poster

P397. Peripheral Mechanisms of Neuropathic Pain

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Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P397.11

Topic: D.03. Somatosensation – Pain

Title: Alterations in peripheral and central nociceptive signaling circuits during HIV/SIV infection contribute to the development of HIV-associated chronic pain

Authors: *R. WARFIELD, J. ROBINSON, M. SMITH, T. BURDO;
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Abstract: With the development of combined anti-retroviral therapy (ART), human immunodeficiency virus (HIV)-associated comorbidities, including development of chronic pain related to HIV-associated distal sensory polyneuropathy (HIV-DSP), has remained prevalent. Using a simian immunodeficiency virus (SIV)-infected rhesus macaque model (N=18) we examined potential mechanisms of peripheral and central sensitization in SIV/HIV-associated chronic pain. Elevated macrophage (CD68+) and T cell (CD3+) numbers were seen in the lumbar spinal cord of SIV-infected (SIV+) macaques (N=6) compared to uninfected animals (N=6) and did not fully return to uninfected levels in the SIV+/ART treated animals (N=6). Thus, these data indicate spinal neuroinflammation occurs in SIV and persists with ART. Similarly, in regard to the dorsal root ganglia (DRG), we have previously shown atrophy in nociceptive neurons is associated with increased inflammatory monocyte traffic to the DRG during SIV infection where neuroinflammation remains despite ART. Here, we demonstrate a significant increase ($P \leq 0.01$) in expression of nociceptive ion channels; transient receptor potential vanilloid (TRPV) and anykrin (TRPA) in the DRGs of SIV+/ART treated rhesus macaques compared to SIV+/ART-naïve group. Nociceptor sensitization in the DRG and central nervous system inflammation can contribute to the development of chronic neuropathy through the release brain derived neurotrophic factor (BDNF) into the dorsal horn. Our data identifies a significant upregulation of the precursor form of BDNF, proBDNF, ($P \leq 0.01$) and not the mature, mBDNF, within the DRGs of SIV+/ART treated macaques. These data indicate a shift in the overall processing or release of BDNF from the primary afferents in the DRGs of SIV+/ART treated animals. To study the mechanism by which alterations in nociceptive ion channels and the pro-/mBDNF ratio within the DRG may affect peripheral sensitization, we have established and characterized a human induced pluripotent stem cell (hPSC)-derived peripheral sensory neuron (PSN) model. We found that sensory neurons derived from both male and female hIPSCs expressed pan neuronal marker, beta 3 tubulin, and peripheral neuron marker, peripherin. The hIPSC PSN expressed both, TRPV and TRPA, and neurotrophin, BDNF, confirmed by both immunocytochemistry and RT-qPCR. In conclusion, we identified neuroinflammation in the spinal cord as a potential mechanism for central sensitization in SIV. Additionally, alterations in nociceptive ion channels and the pro-/mBDNF ratio within the DRG may contribute to peripheral sensitization in HIV-associated chronic pain.

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Poster

P397. Peripheral Mechanisms of Neuropathic Pain

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P397.12

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Sigma 1 antagonist haloperidol reduces tolerance induced by morphine in rats with neuropathic pain

Authors: *L. C. MENA-VALDÉS, Y. BLANCO-HERNÁNDEZ, F. J. LOPEZ-MUÑOZ; Cinvestav-Unidad Coapa, Ciudad de Mexico, Mexico

Abstract: Haloperidol (Hal) is a butyrophenone prototype widely used as an antipsychotic agent. Recently, some authors have described the anti-nociceptive effects of Hal mediated by sigma-1 receptor (S1R) antagonism. Morphine (Mor) is currently used in neuropathic pain (NP) therapy; however, there is increasing evidence that opioid therapy might be associated not only with the development of tolerance, but also with increased pain sensitivity. This work evaluated the interaction between haloperidol and morphine on tolerance induced by morphine in rats with neuropathic pain. Methods: Male Wistar rats (220-250 g) from Cinvestav-Coapa bioterim were used for experiments. The chronic constriction injury (CCI) model was developed to induce NP. The effect of Hal as an S1R antagonist on Mor-induced tolerance was evaluated in experiments conducted at 7, 10 or 14 days. The anti-allodynic effects of repeated administrations (twice/day) of Hal (0.01778 mg/kg), Mor (0.0316 mg/kg) and their combination were evaluated by the cold allodynia test. The effects of sigma-1 antagonist BD-1063, the sigma-1 agonist PRE-084, and the opioid agonist naloxone were used in the tolerance protocols developed. Results: Animals administered in repeated doses of Mor 0.0316 mg/kg presented tolerance to opioids after 5 days of treatment in the 3 protocols evaluated, at 7,10 and 14 days. Although in the animals that received the combination, tolerance was delayed until day 8. PRE-084 and naloxone administered on day 5 in animals treated with the combination resulted in a blockade of its anti-allodynic effects. Conclusions: The interaction between sigma-1 receptor and the opioid system opens a window in neuropathic pain treatment. This study showed that haloperidol potentiates the antinociceptive effects of morphine and, in addition, modulates the tolerance induced by the opioid.

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Poster

P397. Peripheral Mechanisms of Neuropathic Pain

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Program #/Poster #: P397.13

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Irish Research Council. Laureate Award (IRCLA/2017/78).

Title: Sex-dependent modulation of nociceptive behavior through the inhibition of fatty acid amide hydrolase and monoacylglycerol lipase in rats following peripheral neuropathy

Authors: *L. BOULLON[1,2,3,4], S. LEAHY[1], C. CALITZ[1], D. FINN[1,2,3,4], A. LLORENTE-BERZAL[1,2,3,4];
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Abstract: Several reports have demonstrated involvement of the endocannabinoid system (ECS) in the regulation of nociceptive processing. In particular, pharmacological modulation of the ECS in the periaqueductal grey (PAG) induces antinociceptive effects though the inhibition of neurotransmission. In this study, we investigated the effects of systemic inhibition of the anandamide-catabolizing enzyme fatty acid amide hydrolase (FAAH) and the 2-arachidonylglycerol (2-AG)-catabolizing enzyme monoacylglycerol lipase (MGL) in male and female rats following peripheral nerve injury. Administration of the FAAH and MGL inhibitors, URB597 (0.3mg/kg; i.p.) and MJN110 (1mg/kg; i.p.) respectively, commenced 7 days post-surgery (Sham/Spared Nerve Injury (SNI)) with rats receiving one injection/day for 15 days. Von Frey and acetone drop tests evaluated the effect of vehicle/URB597/MJN110 treatment on SNI-induced mechanical and cold hypersensitivity. *Post-mortem* analysis of the PAG investigated endocannabinoid (AEA, 2-AG) and related *N*-acylethanolamine (PEA, OEA) levels using liquid chromatography tandem to mass spectrometry. Furthermore, FAAH, MGL, CB_1-, and CB_2-receptor mRNA levels were measured using RT-qPCR. Nerve injury induced mechanical and cold hypersensitivity in male and female animals, but to a significantly greater extent in in females as previously described (Boullon et al., 2021). Chronic administration of URB597 increased mechanical paw withdrawal threshold (i.e. reduced mechanical hypersensitivity) of female-SNI but not male-SNI rats. Similarly, reduction of acetone-induced nociceptive responses was only observed in URB597 treated female-SNI animals. MJN110 had no significant effect on SNI-induced mechanical or cold hypersensitivity; however, it did reduce paw withdrawal threshold in male-Sham rats. MJN110-treated animals had higher levels of 2-AG in the PAG, compared with vehicle-treated controls. URB597 administration had no effect on AEA levels in the PAG, however it did significantly increase PEA and OEA levels in both sexes. No differences were observed in CB_1, CB_2, FAAH or MGL mRNA levels in the PAG between the groups. These data provide evidence for sexual dimorphism in the antinociceptive effects of FAAH inhibition, which were evident in female rats but not male rats following SNI. The sex-dependent effects of FAAH inhibition on nociceptive were not associated with sex-dependent alterations in components of the endocannabinoid system in the PAG.

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Poster

P445. Auditory Processing: Attention, Arousal, and Neuromodulation

Location: Virtual Only

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Program #/Poster #: P445.01

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant DC016746
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NIH NRSA Fellowship F30DCDC018214

Title: Dopaminergic modulation in auditory decision-making.

Authors: *A. P. F. CHEN[1,2], J. MALGADY[1], L. CHEN[1], K. SHI[1], A. ZETO[1], E. CHENG[1], J. L. PLOTKIN[1], S. GE[1], Q. XIONG[1];
[1]Dept. of Neurobio. and Behavior, [2]Med. Scientist Training Program, Stony Brook Univ., Stony Brook, NY

Abstract: The basal ganglia are implicated in perceptual decision-making, incorporating sensory stimuli and action-outcome encoding. The auditory striatum, a sensory portion of the basal ganglia, is critical for transforming auditory stimuli into ongoing actions. We investigated how dopamine (DA) regulates auditory striatal activity in a frequency discrimination task. Viral retrograde mapping demonstrates that the lateral portion of the substantia nigra (SN) is the primary DA input to the auditory striatum. Cue-locked optogenetic inhibition of this population impaired auditory choice performance on a trial-by-trial basis. Given this impairment, we next investigated how SN activity regulates auditory choice and whether it is important for auditory striatal neuron task engagement. Using ex vivo physiology, optogenetic, and chemogenetic methods, we show that SN activity is critical for regulating auditory striatal DA levels, striatal activity, and auditory task performance. Our study uncovers a phasic mechanism of how the nigrostriatal system regulates auditory decisions, by stably modulating ongoing perceptual performance.

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Poster

P445. Auditory Processing: Attention, Arousal, and Neuromodulation

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Program #/Poster #: P445.02

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant R01DC018650
NIH Grant R00DC015014

Title: Role of the cholinergic system in early sensorimotor acquisition

Authors: *S. E. ELNOZAHY[1], J. LAWLOR[1], F. ZHU[1], T. RAAM[1], A. WANG[1], K. KUCHIBHOTLA[2];
[2]Psychological and Brain Sciences, Neurosci., [1]Johns Hopkins Univ., Baltimore, MD

Abstract: Acquisition of sensorimotor learning in a go/no-go task is much faster and more stereotyped than previously considered (Kuchibhotla et al., 2019). We trained mice to respond to one tone for a water reward (S+) and withhold from responding to another (S-). We interleaved reinforced trials with those where reinforcement was absent ("probe"). Early in learning, animals discriminated between S+ and S- in probe but not reinforced trials. This unmasked a rapid acquisition phase of learning followed by a slower phase for reinforcement, termed 'expression'. What role does neuromodulation play in task acquisition? Prior work has pointed to cholinergic projections from the basal forebrain as a mediator of auditory cortex plasticity. Here, we test the hypothesis that phasic, cholinergic neuromodulation provides a 'teaching signal' that drives primary auditory cortex (A1), and links stimuli with reinforcement. We exploit our behavioral approach and combine this with two-photon imaging of cholinergic activity in A1 during discrimination learning. We report robust stimulus-evoked cholinergic activity to both S+ and S- throughout learning. While this activity mildly habituates in a passive control, in behaving animals the S+ and S- stimulus-evoked activity is enhanced (S+: duration, S-: amplitude and duration) during early learning, on a timescale matching acquisition of the task. Additionally, we test the hypothesis that phasic, cholinergic neuromodulation impacts the rate of task acquisition. We bilaterally express ChR2 in cholinergic neurons within the basal forebrain of ChAT-cre mice and activate these neurons on both S+ and S- trials throughout learning. Probe trials, lacking optogenetic activation, serve as a readout for task acquisition rates. By phasically stimulating cholinergic neurons in the reinforced context, animals acquired the task faster than control groups. This improvement in acquisition rates early in learning is driven largely by a reduction in false alarm rate during probe trials, indicating that phasic activation of the basal forebrain accelerates response inhibition. These results suggest that phasic bursts of acetylcholine, projecting widely to cortical regions, directly impact the rate of discrimination learning.

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Poster

P445. Auditory Processing: Attention, Arousal, and Neuromodulation

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P445.03

Topic: D.06. Auditory & Vestibular Systems

Title: Optogenetics manipulation of GABAergic neurons in the posterior Nucleus Basalis of Meynert modulates auditory information perception.

Authors: *K. THOMAS, H. AZIMI, M. SANDERS, M. HARVEY, G. RAINER;
Univ. de Fribourg, Fribourg, Switzerland

Abstract: The posterior Nucleus Basalis of Meynert (pNB) cholinergic projections to the auditory thalamus and cortex have been extensively documented. However, pNB also contains a population of GABAergic projection neurons, which have been much less studied. Here, we investigate how optogenetic up- und downregulation of GABAergic pNB neurons impacts performance of an auditory detection task using PV-Arch, PV-Chr2 and mDlx-Chr2 constructs. We adapted an auditory detection paradigm, such that freely moving rats were trained to hold down a lever and release it upon the presentation of a narrow bandpass-filtered auditory target stimulus presented with variable amplitudes in the presence of a broad bandpass-filtered masking noise. Spiking activity in auditory primary cortex (A1) and Medial Geniculate Nucleus (MGN) to the target was positively correlated with target amplitude, and significantly attenuated in the presence of the masking noise. Rats acquired the task in about 4 weeks, as we verified using the distribution of lever-holding duration during each session. Behavioral detection performance was closely related to target amplitude, consistent with neural recording data. For functional validation, we demonstrated pulse entrained spiking activity in pNB in response to 40Hz light pulses using blue 473µm light, as well as suppression of pNB spiking during continuous yellow 593µm light in animals transfected with PV Cre-locked AAV5-Chr2 or AAV5-Arch3 constructs respectively. We found specific inhibition of PV GABAergic neurons impaired task performance and increased reaction times for low amplitude targets. Specific activation of pNB PV cells, produced weak effects that were limited to a slight decrease in performance for high amplitude targets. Non subtype-specific activation of the GABAergic population, using a mDlx-Chr2 viral construct, produced general faster reaction times, as well as frequency-specific improvement in detection task performance for low amplitude targets correlated with task engagement. Our findings suggest that under- as well as overactivation of pNB PV neurons is detrimental for detecting an auditory target in the presence of noise. While pNB PV under-activation impairs attentional filtering for low amplitude targets, overactivation reduces performance for high amplitude targets consistent with a more general withdrawal of task resources. Behavioral changes observed following activation of the non-specific pNB GABAergic population points towards an involvement of non-PV inhibitory circuits, i.e. the somatostatin population, in modulating attention in the auditory pathway.

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Poster

P445. Auditory Processing: Attention, Arousal, and Neuromodulation

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Program #/Poster #: P445.04

Topic: D.06. Auditory & Vestibular Systems

Support: NIH NIDCD R01DC017797
NIH NIDCD R03DC015618

Title: Basal forebrain cholinergic neuronal activity across attentional and arousal states during auditory go/no-go behavior

Authors: E. HANSON[1,3], J. W. DE GEE[2,3], M. THOMPSON[2,3], **K. JASPE**[2,3], B. R. ARENKIEL[1,2,3], M. J. MCGINLEY[2,3,4];
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Abstract: Behavioral state characteristics like attention and arousal potently impact numerous cognitive functions, including sensory perception and decision making. Effects of attention and arousal in sensory and decision circuits are mediated, in part, by cholinergic neuromodulation originating in the basal forebrain. However, the precise computational roles of basal forebrain cholinergic signaling in mediating effects of arousal and attention remain open questions. Here we describe an approach that combines a novel auditory go/no-go discrimination task, which requires different degrees of attentional effort, with fiber photometry and pupillometry to investigate the role of basal forebrain cholinergic signaling in attention and arousal-linked modulation of decision-making. In this task, reward size is varied in blocks, allowing us to control the utility of attention and thus manipulate the degree of expended attentional effort. We assay arousal using pupil diameter, and attentional effort via reward block and associated computational measures of behavioral performance changes. This approach allows us to dissociate the effects of arousal and attentional effort on behavior and elucidate the neural underpinnings. To determine the role of basal forebrain cholinergic signaling in task performance across attention and arousal states in the attentional effort task, we use fiber photometry to record from basal forebrain cholinergic neurons in the horizontal limb of the diagonal band of Broca during the task performance. We then correlate basal forebrain cholinergic neuronal activity with pupil diameter, task parameters (reward size and trial timing) and behavior (licking, reward consumption, and running) by applying signal detection theory, drift diffusion modeling, and regression approaches. Ultimately, this approach will allow us to examine precisely when and to what extent basal forebrain cholinergic neurons respond to task-evoked changes in attention, arousal, and reward expectation, and how they influence sensory discrimination and decision-making processes.

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Poster

P445. Auditory Processing: Attention, Arousal, and Neuromodulation

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P445.05

Topic: D.06. Auditory & Vestibular Systems

Title: Identifying conscious perceptual state in mice using a lick-left/lick-right auditory discrimination task

Authors: *S. H. MCGILL[1], C. W. ZHAO[3], L.-A. SIEU[5], T. NGUYEN[4], Q. PERRENOUD[7], J. A. CARDIN[2], H. BLUMENFELD[6];
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Abstract: Research into conscious perception has revealed that a rapid (within one second), stereotyped pattern of neuronal activity changes in both cortical and subcortical areas of the brain occurs following presentation of perceived stimuli but not following identical unperceived stimuli. These and almost all studies conducted on conscious perception have been performed in humans, where methods for establishing causal relationships between neuronal activity and behavior are limited. Mice exhibit many of the same behaviors evocative of conscious perception in humans including selective attention and decision making informed by external stimuli, but as a model have access to robust methods for recording and manipulating neuronal activity. To establish a model for studying conscious perception in mice, we have developed a paradigm for training mice to self-report perception or non-perception of an auditory stimulus within a lick-left/lick-right auditory discrimination task. Adult c57bl6 mice were surgically implanted with a steel headplate and water restricted following recovery. The mice were head-fixed above a running wheel flanked by speakers and presented with two water spouts. Mice were initially trained to lick one spout in response to a primary auditory stimulus, and the other in response to a secondary stimulus following presentation of a light. The spout associated with each sound was randomized for each mouse. The secondary stimulus was then titrated in volume to associate the absence of a stimulus following the light with the secondary stimulus' spout. Mice exhibited lick responses discriminating the presence and absence of an auditory stimulus with an average accuracy of 71% across all sessions. The mice achieved an accuracy significantly greater than chance within 37 sessions or 46 days. These results suggest mice can be trained to provide a behavioral response that distinguishes their perception or non-perception of a stimulus. Using this behavioral task, future electrophysiological or photometric assays may provide insight into mouse brain activity during conscious perception, and optogenetic manipulations may allow causal relationships between neuronal responses and conscious perception to be established.

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Poster

P445. Auditory Processing: Attention, Arousal, and Neuromodulation

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Program #/Poster #: P445.06

Topic: D.06. Auditory & Vestibular Systems

Support: NIH NIDCD R01DC017797
NIH NIDCD R03DC015618

Title: Adaptive increases in attentional effort manifest as suppressed exploratory behavior and reduced physiological arousal

Authors: *J. DE GEE[1], Z. H. MRIDHA[1], M. HUDSON[1], Y. SHI[1], H. RAMSAYWAK[1], S. SMITH[1], N. KAREDIYA[1], M. THOMPSON[1], K. JASPE[1], W. ZHANG[1], M. J. MCGINLEY[2]; [2]Neurosci., [1]Baylor Col. of Med., Houston, TX

Abstract: Depending on the current estimate of a task's utility, one's goal might be to harvest as much reward as possible (exploitation) or disengage from the task to seek alternatives (exploration). One prominent theory argues that increased neuromodulation promotes a shift from exploitation to exploration (Aston-Jones & Cohen, Annu. Rev. Neurosci, 2005). Another prominent theory argues that increased neuromodulation mediates attention (Hasselmo & McGaughy, Progress in Brain Research, 2004). Experimental data constraining and arbitrating between such theories is limited, due to the difficulty to assay and manipulate neuromodulatory systems in behaving animals.

To study the physiological signals that implement attention allocation and adjustments of the exploration-exploration trade-off, we developed an "attentional effort task" for head-fixed mice. In the task, mice lick for sugar-water reward upon detection of temporally unpredictable coherence in a sustained tone-cloud. We manipulate task utility by changing the reward size in blocks of trials. Thus, mice should expend more attentional effort (exploit more) in high reward blocks and disengage more in low reward blocks. We simultaneously recorded pupil size, walking speed, and two-photon images in auditory cortex. We imaged either acetylcholine via $GRAB_{ACh}$ (GPCR-based sensor), cholinergic axons, or projection-specific local neuronal population activity.

Here, we report behavioral and physiological signatures of adaptive shifts in behavior in >75 mice. First, mice better detected the weak sensory signal in the high vs low reward context, indicating that increased task-utility caused an increase in the efficacy of exploitation. Second, in both the high and low reward context, pre-trial baseline pupil size and walking speed were negatively correlated with perceptual sensitivity, and bias had an inverted-U dependence on pupil size, indicating that a low to moderate arousal state is optimal for the task. Pre-trial baseline pupil size and walking speed were higher in the low reward blocks, particularly after

false alarms, indicating that mice are overly aroused and engage in exploratory behaviors when task utility is low. Finally, acetylcholine concentration phasically increased after correct and incorrect licks, in a reward-context dependent manner.
In sum, we find that (pupil-linked) arousal and cholinergic signaling underlie adjustments of the exploration-exploration trade-off in mice. In ongoing work, we are determining the roles of frontal-sensory interactions and other neuromodulatory systems in mediating these adaptive shifts in attentional state.

Disclosures: J. de Gee: None. **Z.H. Mridha:** None. **M. Hudson:** None. **Y. Shi:** None. **H. Ramsaywak:** None. **S. Smith:** None. **N. Karediya:** None. **M. Thompson:** None. **K. Jaspe:** None. **W. Zhang:** None. **M.J. McGinley:** None.

Poster

P445. Auditory Processing: Attention, Arousal, and Neuromodulation

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #:

Topic: D.06. Auditory & Vestibular Systems

Support: NIH NIDCD R03DC015618
NIH NIDCD R01DC017797

Title: Rapid and adaptive shifts in mouse decision-making to changes in task utility

Authors: J. DE GEE[1,2], ***Z. MRIDHA**[1,2], M. HUDSON[1,2], Y. SHI[1,2], H. RAMSAYWAK[1,2], S. SMITH[1,2], N. KAREDIYA[1,2], M. THOMPSON[1,2], K. JASPE[1,2], A. BANTA[1,2,3], W. ZHANG[1,2], M. J. MCGINLEY[1,2,3];
[1]Neurosci., Baylor Col. of Med., Houston, TX; [2]Duncan Neurolog. Res. Inst., Texas Children's Hosp., Houston, TX; [3]Electrical and Computer Engin., Rice Univ., Houston, TX

Abstract: The brain has a remarkable capacity to adaptively shift processing to support a diverse array of behavioral goals, contextual demands, and environmental changes. In sensory domains, the brain can enhance the processing of difficult-to-perceive stimuli when they are important (Kahneman, 1973; Miller and Cohen, 2001) such as when correct discrimination leads to a large reward. Matching effort to potential gains is a key feature of adaptive behavior and must be re-continuously calibrated. To study if and how mice adapt processing to momentary environmental conditions, we have developed an “attentional effort task” for head-fixed mice.
In the attentional effort task, mice learn to lick for sugar-water reward to report detection of the unpredictable emergence of temporal coherence in an ongoing tone-cloud. This acoustic signal is analogous to coherent motion in common visual tasks. To detect all weak-coherence signals, mice would need to sustain an infeasibly high level of attentional effort across the 90-minute sessions. Thus to probe adaptive effort allocation, we switched the sugar-water reward size

(droplet volume) between high and low values in blocks of 60 trials. Thus, mice should expend more attentional effort in high reward blocks.
Here, we report behavioral and algorithmic signatures of rapid and adaptive shifts in attentional effort allocation in >75 mice. First, mice exhibited at least five attentional effort shifts within a single session, which were tightly time-locked to the switches in reward context. Second, increased attentional effort in high reward blocks manifested as improved detection of the weak signals (higher d'). Third, this effect was strongest for signals that occurred relatively late in the trial, indicating that attentional effort in the high vs low reward context was not only higher but also more sustained. Fourth, fitting reaction time data with an accumulation-to-bound model of decision-making revealed that in high reward blocks mice integrated the weak sensory evidence more efficiently within trials (higher drift rate) and more reliably across trials (lower lapse rate / lower drift rate variability).
In sum, we find that mice adapt their allocation of cognitive resources to changes in task utility. In ongoing 2-photon imaging work, we are determining the roles of frontal-sensory interactions and neuromodulatory signals in mediating these shifts in overt behavior.
Kahneman (1973). Attention and effort. Prentice-Hall.
Miller and Cohen (2001). An Integrative Theory of Prefrontal Cortex Function. Annual Review of Neuroscience, 24(1), 167-202.

Disclosures: J. de Gee: None. **Z. Mridha:** None. **M. Hudson:** None. **Y. Shi:** None. **H. Ramsaywak:** None. **S. Smith:** None. **N. Karediya:** None. **M. Thompson:** None. **K. Jaspe:** None. **A. Banta:** None. **W. Zhang:** None. **M.J. McGinley:** None.

Poster

P454. Retinal Circuitry I

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P454.01

Topic: D.07. Vision

Support: DFG BE 5601/2-1
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BMBF Bernstein Center for Computational Neuroscience (01GQ1002)
BMBF Tübingen AI Center to PB (01IS18039A)
Christiane Nuesslein-Volhard-Stiftung

Title: Center-surround interactions underlie bipolar cell motion sensing in the mouse retina

Authors: S. STRAUSS[1,2,3,4], M. M. KORYMPIDOU[1,2,4], Y. RAN[1,2,4], K. FRANKE[1,2,3], T. SCHUBERT[1,2], T. BADEN[1,5], P. BERENS[1,2,3], T. EULER[1,2,3], ***A. VLASITS**[1,2,3];

[1]Inst. for Ophthalmic Res., [2]Ctr. for Integrative Neurosci., [3]Bernstein Ctr. for Computat. Neurosci., [4]Grad. Sch. of Neurosci., Univ. of Tuebingen, Tuebingen, Germany; [5]Sch. of Life Sci., Univ. of Sussex, Brighton, United Kingdom

Abstract: Motion is a critical aspect of vision. We studied the representation of motion in mouse retinal bipolar cells and found, surprisingly, that some bipolar cells possess motion-sensing capabilities that rely on their center-surround receptive fields. Using a glutamate sensor, we directly observed motion-sensitive bipolar cell synaptic output, which was strongest for local motion and dependent on the motion's origin. We characterized bipolar cell receptive fields and found that there are motion and non-motion sensitive bipolar cell clusters, with the majority of clusters being motion sensitive. This motion sensitivity allows bipolar cells to respond in a selective manner to looming motion and directional motion. Motion sensitive bipolar cell clusters were aligned with anatomical bipolar cell types, and were found throughout the sublayers of the inner plexiform layer. Next, we used these bipolar cell receptive fields along with connectomics to design biophysical models of downstream cells in the direction selective circuit. The models demonstrated that bipolar cells pass motion-sensitive excitation to starburst amacrine cells through direction-specific signals mediated by bipolar cells' center-surround receptive field structure. We confirmed some of these model predictions experimentally using 2-photon imaging of calcium sensors expressed in populations of starburst amacrine cells. As bipolar cells provide excitation to most amacrine and ganglion cells, their motion sensitivity may contribute to motion processing throughout the visual system.

Disclosures: S. Strauss: None. **M.M. Korympidou:** None. **Y. Ran:** None. **K. Franke:** None. **T. Schubert:** None. **T. Baden:** None. **P. Berens:** None. **T. Euler:** None. **A. Vlasits:** None.

Poster

P454. Retinal Circuitry I

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Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P454.02

Topic: D.07. Vision

Support: Klingenstein-Simons Fellowship in the Neurosciences
NIH New Innovator Award (DP2 EY027983)
Sloan Research Fellowship

Title: Visual circuits underlying threat anticipation

Authors: *M. L. ARANDA, E. MIN, L. LIU, T. M. SCHMIDT;
Northwestern Univ., Evanston, IL

Abstract: There is a clear evolutionary survival advantage in detecting the presence of threats in an animal's environment. The visual system is one of the primary senses used by mammals to respond to and detect changes in the environment. Moreover, it is crucial that animals are able to anticipate potential dangers in their environment. However, whether and how the visual system may influence these pathways is not fully understood. In this work, we tested the hypothesis that the melanopsin-expressing, intrinsically photosensitive retinal ganglion cells (ipRGCs) are critical for the ability to anticipate the future appearance of a previously experienced threat. To do this, we developed a novel Visual Threat Anticipation paradigm in which we exposed control and melanopsin knock-out (MKO) mice to a threatening, "looming" stimulus. After two days, we measured the degree to which mice anticipate the appearance of that same threatening, looming stimulus in the identical context. Control mice readily learned to anticipate the appearance of this threatening stimulus. Surprisingly, we found that male, but not female, MKO animals lacked threat anticipation, indicating that ipRGCs are required for this behavior. Behavioral measures of vision and anxiety indicated that these deficits were not the result of altered general anxiety or visual deficits. In order to describe this novel circuit, we analyzed c-Fos expression in our Visual Threat Anticipation paradigm, and identified the Perihabenular Region (PHb) and the Nucleus Accumbens (NAcc) as potential candidate brain regions for driving visual threat anticipation. Using choleric toxin β-subunit tracer and Cre-dependent viruses, we observed that ipRGCs project to the PHb, which in turn sends axons to the NAcc. In order to test the functional sufficiency of ipRGC-PHb circuit in visual threat anticipation, we chemogenetically excited PHb-projecting ipRGCs in MKO mice. We found that excitation of PHb-projecting ipRGCs rescued the deficits we observed in threat anticipation. We next tested whether GABAergic neurons in the PHb are necessary for visual threat anticipation by chemogenetically silencing this population. For that we injected Cre-dependent Gi-DREADDs in PHb from $Gad2^{Cre/+}$ and we found that male mice showed reduced visual threat anticipation. Overall, our results suggest that an ipRGC-PHb-NAcc circuit drives visual threat anticipation.

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

Poster

P454. Retinal Circuitry I

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P454.03

Topic: D.07. Vision

Support: R01EY019498
R01EY013528
P30EY003176
NSF GRFP
K99EY030909

Title: Identification and testing of candidate molecular factors that mediate asymmetric inhibitory connectivity in the direction-selective retinal circuit

Authors: *J. TWORIG[1], A. TIRIAC[1], K. BISTRONG[2], R. MORRIE[1], M. FELLER[1];
[1]Mol. and Cell Biol., [2]Helen Wills Neurosci. Inst., Univ. of California, Berkeley, Berkeley, CA

Abstract: Direction-selectivity in the retina is mediated by asymmetric inhibitory connections between starburst amacrine cells (SACs) and direction-selective ganglion cells (DSGCs). This wiring occurs in a DSGC subtype-specific manner, such that individual SAC dendrites preferentially synapse onto DSGCs that are tuned to stimuli moving antiparallel to the SAC dendrites[1]. Thus, any given DSGC subtype receives input from SAC dendrites that are oriented in that DSGC's null direction. The molecular basis of subtype-specific SAC-DSGC wiring is unknown, but it is likely that DSGCs tuned to motion in different axes express distinct markers that permit synaptogenesis with SAC dendrites oriented in a certain direction. To test this, we have performed RNA sequencing on isolated ON-OFF DSGC populations tuned for either nasal or ventral motion. Differential expression analysis revealed a variety of candidate genes with known functions in cell adhesion or synaptogenesis. We validated expression of several candidates using fluorescent in situ hybridization and immunohistochemistry. We will report progress on testing the role of one candidate, the inhibitory synaptic organizer cerebellin-4 (Cbln4), in the development of direction-selective tuning.
1. Briggman, K., Helmstaedter, M. & Denk, W. Wiring specificity in the direction-selectivity circuit of the retina. *Nature* **471,** 183-188 (2011). https://doi.org/10.1038/nature09818

Disclosures: J. Tworig: None. **A. Tiriac:** None. **K. Bistrong:** None. **R. Morrie:** None. **M. Feller:** None.

Poster

P454. Retinal Circuitry I

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P454.04

Topic: D.07. Vision

Support: NIH grant EY030841

Title: Novel object detection and multiplexed motion representation in retinal bipolar cells

Authors: J. A. GAYNES, S. A. BUDOFF, M. J. GRYBKO, J. B. HUNT, *A. POLEG-POLSKY;
Univ. of Colorado Sch. of Med., Aurora, CO

Abstract: Antagonistic interactions between the center and surround receptive field (RF) components lie at the heart of the computations performed in the visual system. Center-surround

RFs are thought to enhance responses to spatial contrasts (i.e., edges), but how they contribute to motion processing is unknown. Here, we addressed this question in retinal bipolar cells, the first visual neuron with classic center-surround interactions. We found that bipolar glutamate release emphasizes objects that emerge in the RF; their responses to continuous motion are smaller, slower, and cannot be predicted by signals elicited by stationary stimuli. The alteration in signal dynamics induced by novel objects dwarfs the enhancement of spatial edges and can be explained by priming of RF surround during continuous motion. These findings echo the salience of human visual perception and demonstrate an unappreciated capacity of the center-surround architecture to facilitate novel object detection and multiplexed encoding of distinct visual data streams.

Disclosures: J.A. Gaynes: None. **S.A. Budoff:** None. **M.J. Grybko:** None. **J.B. Hunt:** None. **A. Poleg-Polsky:** None.

Poster

P454. Retinal Circuitry I

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Program #/Poster #: P454.05

Topic: D.07. Vision

Support: NIH R01EY019498
R01EY013528
P30EY003176
NIH K99EY030909

Title: A role for retinal waves in the development of retinal direction selectivity

Authors: *K. BISTRONG, A. TIRIAC, J. TWORIG, M. B. FELLER;
Univ. of California Berkeley, Berkeley, CA

Abstract: In the developing visual system, neurons in the retina form precise connections to accurately represent features of the visual scene and transduce them into physiologically-relevant outputs. For some features, like direction selectivity, these connections are already established before the onset of vision. The factors that set up these connections during this developmental period remain unknown. During development, the retina exhibits spontaneous waves of neural activity that sweep across its surface. We tested the influence of these retinal waves on the development of the direction selective circuit by characterizing direction selective responses in a mouse model (β2-nAChR-KO) that exhibits perturbed retinal waves. These mice present a severe deficit in direction selective responses to motion along the horizontal axis. This is reminiscent of a recent study where genetic deletion in mice of a gene linked to a human disease model for nystagmus, FRMD7, leads to a severe reduction in horizontal direction selectivity in the mouse

retina. We therefore tested whether FRMD7 expression is influenced by cholinergic retinal waves during development. Using fluorescent in situ hybridization and immunohistochemistry, we find that starburst amacrine cells have normal levels of FRMD7 expression in β2-nAChR-KO mice. This work establishes a role for retinal waves in the development of horizontal direction selectivity.

Disclosures: K. Bistrong: None. **A. Tiriac:** None. **J. Tworig:** None. **M.B. Feller:** None.

Poster

P454. Retinal Circuitry I

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Program #/Poster #: P454.06

Topic: D.07. Vision

Support: R01-EY028542-03

Title: Systematic reduction of the dimensionality of natural scenes allows accurate predictions of retinal ganglion cell spike outputs.

Authors: *J. FREEDLAND, F. M. RIEKE;
Univ. of Washington, Seattle, WA

Abstract: Natural visual scenes contain complex spatial features that are reliably encoded by the mammalian visual system. During early-stage encoding, the retina captures structure in natural scenes through linear and nonlinear spatial integration. Although some of the individual retinal mechanisms that control spatial integration are well-understood, spike predictions to natural scenes remain elusive. This is likely because natural scenes engage multiple circuit mechanisms that interact in poorly understood ways. Here, we identify spatial integration mechanisms relevant for natural scene encoding and use computational modeling to predict spike outputs in primate On- and Off-parasol RGCs. Our approach was to empirically determine how the structure of naturalistic stimuli can be simplified without eliciting major changes to the spike response of parasol RGCs. We were then able to leverage the statistical simplicity of the resulting scenes to investigate spatial mechanisms and predict encoded spike responses.
The simplification of natural stimuli consists of dividing individual frames of natural movies into discrete spatial regions and replacing the spatial structure in each region with a uniform luminance. This luminance value is equal to the linearly-integrated pixel value within each region of the original movie. We find that reducing natural movies to 16-linearly integrated regions is sufficient to describe 80-90% of the structure in the spiking responses of On- and Off-parasol RGCs. We test simplified stimuli by replacing each reduced region with an entirely different image that contains the same integrated luminance. Responses to these spatial metamers are very similar to those to the original natural movie. Finally, we computationally investigate

this 16-dimensional space and observe the underlying center-surround and rectification mechanisms that convert luminance into the cell's 1-dimensional spike output.

Disclosures: J. Freedland: None. **F.M. Rieke:** None.

Poster

P454. Retinal Circuitry I

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Topic: D.07. Vision

Support: F31 EY031985-01
RO1 EY030169
RO1 EY018204
Whitehall Foundation Grant
Research to Prevent Blindness

Title: Metabotropic glutamate receptor 8 creates an unconventional receptive field in a novel interneuron in the mouse retina

Authors: *A. JO[1], S. DENIZ[1], J. XU[2], S. H. DEVRIES[1], Y. ZHU[1];
[1]Ophthalmology, [2]Dept. of Physiol., Northwestern Univ., Chicago, IL

Abstract: In the retina, with the exception of mGluR6, the roles of group-III mGluRs in visual processing remain unclear. Here we used Cre/tTA intersectional mouse genetics to identify a novel amacrine cell type, named Scg2-AC2. Scg2-AC2 is a narrow-field amacrine cell broadly stratified in the ON layer of the inner plexiform layer . However, contradictory to the ON-layer stratification, Scg2-AC2 is activated in the dark but inhibited by the light, as revealed with GCaMP imaging. Furthermore, it exhibits high contrast sensitivity, and shows an unconventional "surround excitation, central inhibition" receptive field. iGluSnFr imaging showed Scg2-AC2 received direct glutamatergic input from ON-bipolar cells in the light. To our surprise, pharmacology experiments revealed that L-AP4 and mGluR8-specific agonist (S) 3,4 DCPG effectively abolished both the dark activation and light inactivation in Scg2-AC2. These results suggest that mGluR8 is expressed in the Scg2-AC2 circuit and mediates the inhibition in light and in response to L-AP4 application. We are currently assessing Single cell RNA-seq and In Situ Hybridization of Scg2-AC2. Our results suggest that its unconventional receptive field, along with its high contrast sensitivity, allow Scg2-AC2 to exert a strong but well-controlled inhibition on the "ON' and/or "ON-OFF" RGCs to enhance visual sensitivity in the dark.

Disclosures: A. Jo: None. **S. Deniz:** None. **J. Xu:** None. **S.H. DeVries:** None. **Y. Zhu:** None.

Poster

P454. Retinal Circuitry I

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P454.08

Topic: D.07. Vision

Support: NIHH R01-EY028188

Title: Toward a comprehensive account of orientation selectivity in the retina

Authors: *M. L. ZIPPERER[1], B. G. BORGHUIS[2];
[1]Univ. of Louisville Anatom. Sci. & Neurobio., Louisville, KY; [2]Anatom. Sci. and Neurobio., Univ. of Louisville, Louisville, KY

Abstract: Retinal Ganglion Cells (RGCs)form functionally distinct ‘channels’that selectively encode features ofthe visual input including direction of motion, contrast, size, and color. A highly conserved visual channelamongst vertebrates conveys orientation selectivity, i.e., the selective firing of neuronal cells in responseto elongated stimuli along a preferred orientation. Orientation selectivity is a critical computation andseveral studies have reported aspects of it, including apparent cell type identity in anatomicalreconstructions, and functional characterization of at least two different identified RGC types. But howcell types in the different studies relate is not well resolved, and the mechanisms that generate theorientation selective responses in mice remain incompletely understood.The goal of this study was to comprehensively characterize Orientation Selective (OS)RGC typesin the mouse retina and to elucidate the mechanisms that contribute to their tuning properties. We usedpopulation calcium imaging and clustering algorithms to identify orientation selective RCGs in retinalexplants. We then targeted these cells for detailed morphological, molecular marker, andelectrophysiological study. Comparison with published data enabled cross-linking morphological,molecular, and functionally defined types of previous studies.Our survey of RGC populations in the Thy1-GcaMP6f mouse line revealed multiple cell typesselective for both horizontal and vertical orientations. Based on data from calcium imaging andelectrophysiological whole-cell recording, the horizontal-tuned cluster appears to comprise at least 3distinct types. Analysis of light-evoked synaptic currents shows one cell with fast transient inhibition andanother with slow tonic inhibition. A third type was distinguished by tuned excitation and inhibition inresponse to a drifting grating stimulus. Sulforhodamine and neurobiotin tracing revealed gap junctioncoupling in select OS cells, which raises the possibility that electrical synapses contribute to their tuning.Based on our morphological data we conclude that one type is the Tbr1-S2 cell described in Liu et al.,(2018)and that this is the same cell type as the 4on type in the eyewire museum classification(www.eyewire.com).This study has identified multiple OSGC types in mouse and provides insight into some of thecircuit-level tuning mechanisms of these cells. We have begun to resolve correspondences betweenreported OS RGCs and incompletely categorized cell types

from previous studies, contributing to a morecomplete understanding of RGC types and their functional role in the mouse retina.

Disclosures: M.L. Zipperer: None. **B.G. Borghuis:** None.

Poster

P454. Retinal Circuitry I

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P454.09

Topic: D.07. Vision

Support: NIH R01EY029227
NIH T32GM132057

Title: Endogenous opioid signaling in the retina modulates sleep/wake behavior

Authors: *C.-T. BEREZIN[1], N. BERGUM[2], K. A. LUCHINI[2], J. VIGH[2];
[1]Cell and Mol. Biol., [2]Biomed. Sci., Colorado State Univ., Fort Collins, CO

Abstract: Light is perhaps the most important environmental factor influencing sleep/wake behavior. In mammals, sleep/wake cycles are set to light/dark cycles in the environment (i.e. photoentrainment) by intrinsically photosensitive retinal ganglion cells (ipRGCs); ipRGCs fire action potentials in response to light which, in nocturnal mice, promotes sleep. Importantly, ipRGC firing is inhibited by μ-opioid receptor (MOR) activation. MOR-selective neuromodulators that alter the activity of ipRGCs can presumably influence light-driven behaviors mediated by ipRGCs. Thus, we hypothesize that the endogenous opioid peptide β-endorphin modulates sleep/wake cycles via MORs on ipRGCs. Our immunohistochemical experiments reveal that there is a statistically significant increase in β-endorphin expression in the retina in nighttime versus daytime, as well as in dark versus light conditions. In addition, telemetric recordings of sleep-wake behavior in transgenic mice which lack functional MORs on ipRGCs show reduced wakefulness during the subjective night compared to wild-type mice. Therefore, we conclude that β-endorphin expression in the retina depends on both circadian timing and light exposure, which is consistent with previous studies showing circadian regulation of β-endorphin expression in the brain. In addition, our data support the hypothesis that endogenous opioid signaling in the retina contributes to healthy sleep-wake cycles. Furthermore, these results suggest that aberrant MOR signaling in ipRGCs might contribute to the sleep disturbances associated with chronic opioid use.

Disclosures: C. Berezin: None. **N. Bergum:** None. **K.A. Luchini:** None. **J. Vigh:** None.

Poster

P454. Retinal Circuitry I

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Program #/Poster #: P454.10

Topic: D.07. Vision

Support: NIH Grant 1F30EY031565
NIH Grant T32GM008152
VitreoRetinal Surgical Foundation fellowship award
NIH Grant 5R01EY031329

Title: Classification of retinal ganglion cells using electrophysiology and functional imaging

Authors: *Z. F. JESSEN, G. W. SCHWARTZ;
Ophthalmology, Northwestern Univ., Chicago, IL

Abstract: Typology of retinal neurons is critical to our understanding of retinal circuitry, yet our knowledge of retinal cell types is incomplete, and typing is used inconsistently across the literature. Typology of retinal ganglion cells (RGCs) has historically relied on functional, transcriptomic, or morphologic approaches, each of which capture a limited amount of inter-type variability relevant to classification. We recently reported a large-scale, intersectional classification of RGCs in which we found that electrophysiological responses to a simple light stimulus were sufficient to separate RGC types with surprising accuracy. Here we present the results of our early attempts to align electrophysiology- and functional imaging- based classifications using a similar approach. Using this alignment we aim to describe how hyperacuity may arise at the level of locally-complete populations of individual RGC types.

Disclosures: Z.F. Jessen: None. **G.W. Schwartz:** None.

Poster

P462. Functional Role of Interneurons in Visual Cortex

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P462.01

Topic: D.07. Vision

Support: NIH NRSA F31

Title: Developmental dysfunction of parvalbumin expressing inhibitory neurons

Authors: *C. WARD, E. SABRI, R. BATISTA-BRITO;
Dominick P. Purpura Dept. of Neurosci., Albert Einstein Col. of Med., Bronx, NY

Abstract: Central to understanding the role of inhibitory neurons in cortical network function, is understanding their contributions through a neurodevelopmental lens. To address this, we disrupted the expression of a critical regulator of neuronal maturation in interneuron precursors. Myocyte enhancer factor-2C (Mef2c) is a transcription factor linked to neurodevelopmental disorders that regulates the expression of genes involved in the differentiation, migration, survival, and synaptic function of excitatory neurons, yet its role in the function of inhibitory neurons is unknown. Among the three major subtypes of cortical inhibitory interneurons, neurons expressing the calcium-binding protein Parvalbumin are highly enriched for Mef2c expression. Parvalbumin-Expressing Inhibitory Interneurons (PV-INs) sample input from large populations of pyramidal cells and synapse onto the soma and axon initial segment, allowing them powerfully to regulate excitatory activity within the cortex. Embryonic removal of Mef2c in PV-IN precursors leads to both signatures of impaired maturation in PV-INs throughout the cortex and a reduction in PV expression within cortical layer IV, the target of sensory inputs from the thalamus. To understand how PV-IN maturation & integration into cortical circuits is affected by loss of Mef2c, we performed awake extracellular electrophysiology recordings within the primary visual cortex across behavioral states. We observed that mutant mice showed altered temporal patterns of network activity, particularly during periods of quiescence, or in the absence of movement. Mutant mice had increased synchronous activity and display hyper-synchronous events. Mutants also showed altered visual responses, including increased noise correlations. Together, our data show that early removal of Mef2c leads to abnormal circuit function. These studies serve as a foundation for examining the contribution of PV-INs toward signatures of altered state-dependent network activity and sensory processing.

Disclosures: C. Ward: None. **E. Sabri:** None. **R. Batista-Brito:** None.

Poster

P462. Functional Role of Interneurons in Visual Cortex

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P462.02

Topic: D.07. Vision

Support: IARPA contract D16PC00004

Title: Diverse inhibitory connectivity from a connectomic census of mouse primary visual cortex

Authors: *C. SCHNEIDER-MIZELL[1], A. L. BODOR[1], F. C. COLLMAN[3], L. ELABBADY[1], M. M. TAKENO[2], J. BUCHANAN[5], R. TORRES[3], S. KINN[3], D. BRITTAIN[3], G. MAHALINGAM[3], D. J. BUMBARGER[2], W. YIN, ESQ[1], S. DORKENWALD[6], N. L.

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Abstract: Mammalian neocortex contains a stunning diversity of inhibitory interneurons, each with distinct morphological, functional, and transcriptomic features. While much progress has been made in studying the connectivity of interneurons at the level of broad molecular sub-classes such as PV, SST, and VIP, each class is known to contain multiple types. It remains difficult to relate these more specific neuronal subtypes to their connectivity. Here, we address this problem using a millimeter-scale electron microscopy volume of mouse visual cortex, with cells and synapses segmented using state-of-the-art machine learning and a highly scalable proofreading framework. To obtain an unbiased population of cortical cells, we generated a columnar "core sample" containing all cells whose nuclei were located within a 100 µm x 100 µm x 700 µm region, spanning from mid-layer 1 to the start of white matter. We found 1343 neurons, 1182 excitatory and 161 inhibitory based on morphological features. In order to understand the distribution of synaptic input in the column, we proofread the dendritic arbors of all columnar neurons and identified dendritic compartments for excitatory cells. Neurons were cell-typed by laminar position, connectivity, and morphological features. The result is a complete input budget based on 4 million synapses across the column, with each categorized by location, postsynaptic cell type, and postsynaptic compartment. In order to measure the morphology and synaptic targeting rules for inhibitory neurons in their local columnar environment, we further extensively proofread the axonal arbors of all inhibitory neurons. Importantly, the size of the volume was such that most proofread arbors were fully contained within the dataset. By comparing the cell-specific patterns of output with the synaptic budget, we not only characterized the outgoing connectivity of each interneuron but also significant deviations from target-specific synapse abundances in the column. In addition to a precise description of the synaptic output of individual cells, our data reveal novel principles of cell-type specific connectivity in cortex.

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Poster

P462. Functional Role of Interneurons in Visual Cortex

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Title: Distinct synaptic connectivity patterns of morphologically-diverse martinotti cells in mouse primary visual cortex

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Abstract: Neuronal cell types can be defined by multiple cellular properties, including morphology, transcriptomic profile, and electrophysiology; however, these modalities may not always agree. Adding synaptic connectivity data can further define a cell type and provide greater insight into that cell's role within the circuit. Martinotti cells (MCs), a class of inhibitory interneurons in the mammalian cortex, have been characterized by the expression of somatostatin (Sst) and axon that elaborates in Layer 1 (L1). The term "Martinotti" likely encompasses multiple cell types as MCs have diverse morphologies, including "T-shape" and "fanning". Furthermore, recent work in the mouse primary visual cortex (VISp) shows several cell types from the Sst subclass with congruent morphology, transcriptomics, and physiology, with distinct axonal arborizations, including L1 innervation (Gouwens & Sorensen et al., 2020). We hypothesize that Martinotti cells with distinct morphologies will also have distinct postsynaptic target distribution patterns.
To test this hypothesis, we completed reconstructions of 11 L4-5 Martinotti (at least 3 cells per distinct morphology) in mouse VISp from a millimeter-scale serial-section electron microscopy (EM) dataset. We proofread both axon and dendrite to remove any erroneous processes and extend processes to a natural end or as far as the data allowed. Skeletons were then extracted from these complete reconstructions and morphological features were calculated from the skeletons. These morphological features were compared with those from VISp Sst+ cells reconstructed from biocytin fills from slice (Gouwens & Sorensen et al., 2020). Additionally, the output synapses from these complete reconstructions were identified by machine-learning (Turner et al., 2020), and a subset were manually confirmed. We identified the postsynaptic cell type and structure (spine vs shaft) for 5% of the output synapses (~70-700 synapses/cell) to determine the postsynaptic target distribution pattern for each cell. From this fraction of synapses, we observe different targeting patterns for these morphologically-distinct sets of neurons.

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Poster

P462. Functional Role of Interneurons in Visual Cortex

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Title: Vip-expressing interneurons in the mouse primary visual cortex control how the arousal state is reflected in the neuronal activity

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Abstract: The mouse primary visual cortex (V1) is the first area where visual information arrives in the neocortex. The cortical circuitry that underlies the sensory processing in V1 is fairly well described in terms of different populations of excitatory and inhibitory neurons residing across the layers of the cortex. Based on our current understanding, inputs from the thalamus mostly target layers 4 and 6 of the sensory cortices, after which information is distributed to the more superficial layers, then is carried to the neurons in layer 5, which in turn send this processed sensory information to other regions. Recent findings have shown that the arousal state of the animal influences the processing of sensory information in cortical sensory areas. However, we don't have a clear picture of the circuitry behind the modulation of sensory information with arousal. Vasoactive Intestinal Polypeptide (VIP)-expressing interneurons reside mainly in superficial layers and receive neuromodulatory and corticocortical connections. These anatomical characteristics put VIP interneurons in a unique position to mediate the effect of arousal on visual information processing. To assess this hypothesis, we silenced the activity of VIP neurons of V1 by using optogenetics in transgenic mice that express inhibitory opsin ArchT exclusively in VIP cells (VIP-cre+/-; Ai40+/−, n=6). During the opto-inhibition of VIP neurons, we recorded the extracellular activity of V1 neurons using a 64-channel linear probe that covers all layers. We used the pupil diameter and the facial movement of the animals to measure the arousal level. We observed that the inhibition of VIP cells slightly decreased the activity across the population both during the low-arousal and high-arousal states ($p=0.037$ and $p=0.005$ respectively) as expected since it is known that VIP cells disinhibit the pyramidal cells. However, the inhibition of VIPs strengthened the level of modulation by arousal. We observed that: 1) 11% of neurons (15/131) showed an enhanced activity during high arousal, and silencing

of VIPs intensified this enhancement (p=0.041). 2) 10% of neurons (14/131) showed a suppressed activity during high arousal, and silencing of VIPs intensified this suppression (p=0.021). Taken together, this data suggests that VIP interneurons are part of the circuitry that controls the transfer of the information about the arousal state to V1. Furthermore, the inactivity of VIPs opens the gate for the influence of arousal state on neuronal activity in V1.

Disclosures: E. Sabri: None. **R. Batista-Brito:** None.

Poster

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Topic: D.07. Vision

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Title: Visual perception depends on the spatial footprint of cortical inhibitory neuron activity

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Abstract: The role of cortical inhibitory neurons during visual perception remains debated. Parvalbumin-expressing (PV) and somatostatin expressing (SST) inhibitory neurons show different spatial preferences for visual stimuli (Adesnik et al., 2012), and provide different spatial spread of inhibition (Kato et al., 2017). It is unknown how the spatial footprint of PV or SST mediated inhibition modulates visual perception. We explored this question by pairing spatiotemporally precise optogenetic activation of PV or SST neurons with simultaneous neural recordings in mice performing a visual contrast detection task.
We trained mice to detect visual stimuli by licking for water (9 mice, 105 sessions). Visual stimuli appeared in blocks of trials across different contrasts in two separate positions: one in central visual space, the other 70° away in peripheral visual space (Speed et al., 2019). Perceptual performance was quantified using signal detection theory. During trials of stimulus detection in the central visual field, PV or SST inhibitory neurons were activated in the part of V1 representing peripheral visual space (~0.8 mm away from V1 site representing central visual space; laser ~0.3 mm full-width half max profile). This allowed us to probe how the spatial spread of inhibition in cortical space affected perception of distant stimulus-evoked activity. PV or SST stimulation exerted unique effects on perceptual performance. SST stimulation largely impaired low contrast stimulus detection but spared high contrast detection, changing the slope

of the psychometric contrast sensitivity curve (~70% decrease in slope; 10% increase in lapse rate). PV stimulation however decreased performance at all contrasts with a smaller change in slope (~35% decrease in slope; ~10% increase in lapse rate).
We next investigated V1 activity during these perceptual changes by performing multisite extracellular recordings during distant optogenetic stimulation in awake mice (n = 6 mice; 28 recordings). During detection of stimuli in the central visual field, distant PV stimulation decreased both excitatory neuron and PV neuron activity in the region of V1 corresponding to the central visual field. At these same sites, distant SST stimulation instead increased SST activity, and also simultaneously decreased PV and excitatory neuron activity. The inhibitory effects of distal SST stimulation on excitatory neurons were twice as large as effects from PV stimulation. These results suggest that cortical inhibitory neuron subtypes drive unique spatial profiles of inhibition that distinctly shape the sensitivity of visual perception.

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Poster

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Title: Chemogenetic suppression of VIP interneurons disrupts top-down alpha-band synchrony during visual oddball paradigm

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Abstract: Temporal synchronization among distributed regions and cell types in the cortex tends to oscillatory in nature. Given the diverse intrinsic properties of individual neurons and synapses, distinct neuro-oscillatory frequency bands may serve to selectively route information processing in cortical networks. Feedback inputs from higher level brain regions are known to modulate sensory processing in lower sensory regions in accord with context and behavioral goals. The interplay between long range connectivity and frequency specific modulation of local cell populations could be a way to encode context and modulate stimulus specific responses in sensory cortex, aligning the status of microcircuits towards a behavioral goal. Interneuron population are specially qualified to be the mediators of long range information processing due

to their inhibitory nature that defines the timing and flow of activity in local circuits.In here, we recorded local field potentials (LFP) from the anterior cingulate cortex (ACa, analog to pre frontal cortex in humans) and primary visual cortex (V1) of male and female mice to explore the oscillatory synchrony between regions during a visual oddball paradigm. The visual oddball paradigm probes the ability of the mice to discriminate between a repetitive stimulus (redundant, shown 87.5% of the time) over stimuli that deviates from it (deviant, shown 12.5% of the time). We found that during the task, ACa and V1 are phase-locked at theta to alpha frequency (6-10hz), showing a high value of oscillatory coherence between both regions. In addition, using optogenetics to rhythmically activate ACa axons in V1, results show an increased activation of VIP interneurons and pyramidal cells and decreased activation of somatostatin (SST) interneurons, specifically when the input was driven at theta to alpha band (6-10hz), but not other frequencies (e.g. delta, beta, or gamma). This suggests that top-down inputs to V1 engage a canonical VIP to SST inhibitory motif in V1 previously reported in the literature.To further test the role of VIP interneurons on the frontal-sensory circuit, we recorded LFP signals from both ACa and V1 while de-activating of VIP cells using chemogenetics during the oddball paradigm (with hm4D(i), n=6 mice) and CNO-only controls (n=7 mice). We found that theta-alpha specific oscillatory coherence significantly decreased after VIP suppression. Further, deviant-stimulus evoked responses in the LFP were also attenuated, suggesting that this manipulation disrupted context processing. The results point to a role of VIP interneurons in mediating theta-alpha synchrony in ACa-V1 circuits during the visual processing of context.

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Poster

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Title: Differential control of pyramidal neuron firing in two visual cortical areas by an elusive inhibitory interneuron

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Abstract: Differential control of pyramidal neuron firing in two visual cortical areas by an elusive inhibitory interneuron

Fani Koukouli[1]*, Martin Montmerle[1]*, Andrea Aguirre[1], Jérémy Peixoto[1], Vikash Choudhary[1], Marcel De Brito Van Velze[1], Camille Allene[1], Pablo Mendéz[3], Giovanni Marsicano[2], Oliver Schlüter[4], Nelson Rebola[1], Alberto Bacci[1#] and Joana Lourenço[1#]
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Fast synaptic GABAergic inhibition shapes both spontaneous and sensory-evoked cortical activity. Cortical inhibitory GABAergic neurons are highly heterogeneous, and this rich diversity of cell types is responsible for specific connectivity patterns with excitatory principal neurons (PNs). The result is an efficient control of cortical activity via a highly specialized division of labor of different interneuron types. In particular, perisomatic inhibition from parvalbumin (PV)-expressing basket cells (BCs) onto PNs orchestrates and sets the dynamic range of cortical networks. Cannabinoid receptor type 1 (CB1)-expressing interneurons also target the perisomatic region of PNs and are known to express several forms of cortical plasticity. However, the connectivity and function of these elusive - yet prominent - neocortical GABAergic cells is unknown. In order to identify the circuit properties of CB1 interneurons, we combined mouse genetics, *in vitro* whole-cell patch clamp recordings and *in vivo* two-photon calcium imaging in awake head-fixed mice. We found that the connectivity pattern of CB1 BCs strongly differs between primary and high-order cortical visual areas. Tonic CB1 signaling constitutively suppresses GABA release from CB1 BCs in the medial secondary visual cortex (V2M), but not primary (V1) visual area. This area-specific connectivity and endocannabinoid-dependent modulation of GABA release from CB1 BCs is responsible for a much higher spontaneous activity of PNs in V2M, as compared to V1 in vivo. In addition,
visual area-specific tonic CB1 signaling modulates PN coordinated activity in V2M but not in V1. Thus, CB1 BCs operate a visual area-dependent dynamic inhibitory control of PNs that will likely play a major role in visual perception.

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Poster

P462. Functional Role of Interneurons in Visual Cortex

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Title: Investigating the role of GABA-mediated visual intracortical inhibition in aging with transcranial magnetic stimulation

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Abstract: Deficits in the inhibitory neurotransmitter γ-aminobutyric acid (GABA) are associated with a number of motor and psychiatric disorders and may also play a role in age-related cognitive decline. However, there are limited ways of probing GABA and inhibitory function in vivo in humans. While Magnetic Resonance Spectroscopy (MRS) can assess the concentration of GABA, this measure may not be indicative of local inhibitory activity. Instead, paired-pulse transcranial magnetic stimulation (ppTMS) allows investigation of inhibitory networks mediated through the GABA-A receptor at the level of interneurons within the primary motor cortex. However, the association between GABAergic inhibition and aging in the visual domain is poorly understood. We recently demonstrated the feasibility, reliability, and optimal stimulation parameters of a ppTMS measure of inhibition by means of short-interval intracortical inhibition of the primary visual cortex (V1). While TMS of the motor cortex elicits measurable muscle-twitches, stimulation of V1 elicits short-lived visual percepts called phosphenes, which can be objectively measured using a tracing paradigm. In the present study, we utilize this new method to investigate visual intracortical inhibition in aging. Younger (18 to 29 years) and older (65+ years) adult participants completed both an MRS session to assess left primary visual GABA concentration and a ppTMS session to assess intracortical inhibition in left V1. Preliminary data in 11 older and 16 younger adults indicate a strong positive relationship between spectroscopy measures of GABA and the ppTMS measure of functional inhibition in V1 ($r = .66$, $p < .001$). We also observed a trend of reduced visual intracortical inhibition with age ($p = .09$). The results demonstrate the possibility of assessing GABAergic function in cortical areas outside of the motor cortex, critical for characterizing the role it plays in both disease and healthy aging.

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Poster

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Title: Exploring the role of interneurons on cortical ensembles

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Abstract: Coordinated neuronal activity is increasingly being considered key for cortical information encoding and processing, as individual neuronal activity seems to be too variable to precisely represent sensory stimuli. Recent work is showing that it is possible to represent sensory information with lower dimensional activity spaces defined by groups of neurons with coordinated activity, called ensembles, extracted from the highly variable and multidimensional excitatory cortical activity. However, the cortex also contains inhibitory interneurons that, although fewer in number, are also essential for normal information processing. The study of interneurons in the cortex has been mostly focused on their effects on the activity of individual excitatory cells but their own coordinated activity and its influence on excitatory ensemble dynamics is still poorly characterized. This is partially due to the greater diversity of interneuronal cell types with different electrophysiological properties, which, with current genetic targeting and identification methods, either decreases the amount of analyzed cells (hampering population analyses) or introduces additional response variability. In our work we set out to explore this by using genetic targeting of interneuron and two photon calcium imaging and holographic optogenetics in mouse primary visual cortex *in vivo* during the presentation of a diverse set of visual stimuli in two ways. First, by recording inhibitory activity in the full interneuronal cortical population, irrespectively or their electrophysiological diversity, we analyze their global activity and extract their population dynamics in combination with excitatory cells. Second, using a recently developed transgenic line to specifically target chandelier cells, a very distinctive and specific interneuronal subtype, we examine and probe their precise role in modulating excitatory cortical activity. Our data shows that a considerable number of interneurons, including chandelier cells, are highly correlated with locomotion and pupil diameter (as a proxy for arousal state). However, we also observed additional structuring of their population dynamics in relation to visual stimulation, with particularly strong correlation between chandelier cells. Additional analysis of the network properties of these populations,

their response to visual stimuli and optogenetic perturbations during perceptual habituation would further reveal the effects of inhibition in the dynamical cortical activity.

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Poster

P462. Functional Role of Interneurons in Visual Cortex

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Topic: D.07. Vision

Title: The role of long-range inhibitory neurons in cortical circuits

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Abstract: Cortical activity is highly dependent on behavioral state of an animal with different states, such as sleep and wake, having profound impact on cognition and cortical processing. The circuitry responsible for sensing behavioral state and modifying cortical activity to generate cortical states remains unknown. Here, we propose that long-range cortical inhibitory neurons in the cortex play a crucial role in the generation of low frequency activity, a hallmark of multiple cortical states. Using an array of complementary techniques, we have begun to characterize this unique cell type. We examine the input and outputs of these long-range inhibitory neurons in mouse primary visual cortex using whole mount light sheet microscopy and monosynaptic rabies tracing. These data show inputs almost exclusively from local cortical neurons and the lateral geniculate nucleus unique from other inhibitory neurons which have connections from many cortical and subcortical structures. While the input is mostly local, outputs span across cortical and subcortical regions. To compliment this, we combine multiple in vivo recording modalities. First, using 2 photon calcium imaging, we show that these cells are active during periods of low arousal states with increased low frequency activity and suppressed by movement. Using electrophysiology and optogenetics, we show that activation of long-range inhibitory neurons leads to increased low-frequency activity. Taken together, these data suggest long-range inhibitory neurons broadly sense local network activity, relaying it to long range targets to temporally synchronize activity during periods of low arousal and increased low frequency activity.

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Poster

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Title: Long term effects of sexual behavior on brain activity in female rats evaluated by manganese-enhanced magnetic resonance imaging (MEMRI)

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Abstract: We used manganese-enhanced magnetic resonance imaging (MEMRI) to evaluate the activation of different brain regions in females that did not and that paced the sexual interaction. Female rats were divided in four groups: 1) control, 2) exposed to a male without mating, and two groups that mated 3) with the male or 4) the female controlling the sexual interaction. The tests were done once a week, for 1 h, for 10 weeks. In session 1, 5 and 10, all females were administrated with 16 mg/kg of manganese chloride ($MnCl_2$) 24 h before testing. Immediately after the tests, subjects were scanned for MEMRI evaluating the olfactory bulb (OB), bed nucleus of the stria terminalis (BNST), amygdala (AMG), the medial preoptic area (MPOA), the ventromedial hypothalamus (VMH), nucleus accumbens (NAcc), the striatum (STR), the hippocampus (Hipp) and the ventral tegmental area (VTA). Consistent with our previous observations we found an increase in signal intensity in most areas evaluated in session 5 in comparison to session 1 in both groups that mated. No differences were observed between females that did not pace the sexual interaction and those that did. When we compared the socio-sexual circuit between the groups we found a significant increase of signal intensity in session 5 in the non-paced mating group against the control group. On the reward circuit, the no paced and paced mating groups had a higher signal intensity compared to the group exposed to the male. Differences across time were found in both circuits in all groups, except for the control. Sessions 5 and 10 had an increased signal intensity compared to their respective session 1. Our results give new insights of how brain activation can be modified across time depending on the duration, quantity and quality of the stimulation that a female receive from a stimulus male.

Disclosures: J. Aguilar Moreno: None. **J. Ortíz:** None. **S. Alcauter:** None. **R.G. Paredes:** None.

Poster

P592. Sexual Behavior

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P592.02

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Director's New Innovator Award conferred to Melissa Warden

Title: Female dorsal raphe serotonin responses to sexual stimuli

Authors: *E. L. TROCONIS, C. SEO, A. GURU, M. R. WARDEN;
Cornell Univ., Ithaca, NY

Abstract: Serotonin—a widely-projecting neuromodulatory system—has been implicated in a variety of emotional brain states and behaviors, including mating. Previous work in both humans and animal models has shown that pharmacological compounds that interact with the serotonin system can modulate female sexual behavior. However, whether female serotonin neurons normally respond to sexual stimuli during mating is unknown. Here, we used fiber photometry to record population calcium activity of dorsal raphe serotonin neurons in the female brain during mating. We found that this population becomes active in the female at the time of ejaculation (GCaMP6, n=14; GFP, n=3; $p<0.001$). To understand which stimuli during ejaculation trigger this neuronal activity in the female, we developed surgical techniques that perturb various aspects of ejaculatory physiology in males with intact mating behavior. First, we tested the role of ejaculatory fluid release into the vaginal canal by redirecting the male urethra to a stoma in the abdominal wall. Then, we tested the role of penis erectile function by partially transecting a pair of muscles that control penis behavior. We found that the female elevated serotonin neuronal activity at ejaculation disappeared only when her mating partner had undergone both modifications in combination (surgery, n=7; control, n=7; $p<0.01$). Moreover, artificial mechanical vaginal stimulation was sufficient to trigger serotonin neuronal activity in the female (GCaMP6, n=6; GFP, n=3; $p<0.05$). Together, these results suggest that both penis behavior and release of ejaculatory fluid inside the vaginal canal are important mechanical stimuli to which female dorsal raphe serotonin neurons respond during ejaculation. Sensing ejaculation may be crucial for regulating various aspects of female sexual behavior and physiology, such as receptivity in subsequent mating encounters and fertility. Overall, our work sheds light on a novel vagina-brain axis that likely plays a critical role in female reproduction.

Disclosures: E.L. Troconis: None. **C. Seo:** None. **A. Guru:** None. **M.R. Warden:** None.

Poster

P592. Sexual Behavior

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P592.03

Topic: F.02. Behavioral Neuroendocrinology

Title: Oxytocin and testosterone shape viewing preference for perinea over faces in potential sexual partners

Authors: Y. JIANG, M. L. PLATT;
Neurosci., Univ. of Pennsylvania, Philadelphia, PA

Abstract: As primates, faces as well as perinea of potential sexual partners naturally demand our attention. Many primates also visually check conspecifics of the same sex for information regarding their dominance and reproductive status. Different hormones may bias our visual attention to different types of displays. Using a free viewing task in which images of monkey faces and perinea were simultaneously displayed, we found that male rhesus macaques showed a significant and consistent preference for perinea over faces, regardless of the sex of the depicted monkey. Male rhesus also universally preferred unobstructed frontal views of faces and perinea of both sexes, possibly due to the richness of visual information presented. In addition, they preferred direct gaze in female faces and open mouths in male faces, suggesting a trade-off between mating interests and monitoring the dominance status of others. Administering both oxytocin and testosterone in male monkeys amplified their innate preference for female but not male perinea, specifically by further dampening attraction to less-preferred faces and increasing attention to more-preferred perinea. Together these results strongly endorse a pre-existing bias in primates to attend to perinea rather than faces, and implicate both oxytocin and testosterone in the process of mate evaluation through visual orienting.

Disclosures: Y. Jiang: None. **M.L. Platt:** None.

Poster

P592. Sexual Behavior

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P592.04

Topic: F.02. Behavioral Neuroendocrinology

Support: University of Kentucky Start Up Funds

Title: Water is necessary for photoperiodic testicular recrudescence in zebra finches but not vocal state change

Authors: *J. SANTOLLO, S. M. FERGUSON, C. E. HARPOLE, B. T. BOWLING, N. R. SOMMERS, C. M. CASSONE;
Univ. of Kentucky, Lexington, KY

Abstract: Zebra finches are considered opportunistic breeders as early reports attributed gonadal recrudescence and the induction of mating behavior to water availability. More recent studies, however, demonstrated a role for photoperiod in regulating zebra finch reproductive behavior. It is not known, however, whether photoperiod and water availability interact to control reproductive function. We, therefore, tested the hypothesis that both long photoperiods and water access are necessary for gonadal recrudescence and vocalizations in male zebra finches. Thirty-nine male zebra finches were divided into two groups that received either *ad libitum* access to water or 0.5 ml of water/ twice per week, while being maintained on a short day (SD) light cycle (06:18 L:D). After four weeks of SD housing, half of the subjects in each fluid access condition were switched to a long day (LD) light cycle (14:10 L:D) for an additional six weeks. Blood samples were taken biweekly, and daily vocalizations and activity were monitored throughout the duration of the study. At the end of the study birds were euthanized, and testes and brains were collected. Analysis of testes weight demonstrated that birds housed under LD with *ad libitum* water access had significantly larger testes compared to all other groups ($p < 0.05$). Locomotor activity and vocalization, however, were greater in LD housed birds but were not influenced by water access ($p < 0.05$). Ongoing studies are examining aromatase and vasotocin expression, as well as morphometric analyses of song nuclei and hypothalamic structures in the brain using cytochrome oxidase histochemistry. These findings are novel in two ways by demonstrating that hydration status gates the ability of photoperiod to induce reproductive capacity and disassociating gonadal function from song vocalizations, an important aspect of songbird mating behavior.

Disclosures: J. Santollo: None. **S.M. Ferguson:** None. **C.E. Harpole:** None. **B.T. Bowling:** None. **N.R. Sommers:** None. **C.M. Cassone:** None.

Poster

P592. Sexual Behavior

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Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P592.05

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant R01NS049488
NIH Grant R01HD104565

Title: Hypothalamic circuit mechanism for temporal control of female sexual behavior

Authors: *S. INOUE[1], R. YANG[1], A. TANTRY[1], C.-H. DAVIS[1], T. YANG[1], J. R. KNOEDLER[1], Y. WEI[1], E. L. ADAMS[1], S. THOMBARE[1], S. R. GOLF[1], R. L. NEVE[2], M. TESSIER-LAVIGNE[1], J. B. DING[1], N. M. SHAH[1];
[1]Stanford Univ., Stanford, CA; [2]Massachusetts Gen. Hosp., Cambridge, MA

Abstract: Behaviors are associated with relevant internal physiological states. In many species, including mice, females dramatically change their sexual behaviors to match their ovulatory status. Females of many species are sexually receptive exclusively during the estrus, ovulatory phase of the estrous cycle, while they are not receptive, and in fact actively rejecting, during other phases. Sex hormones such as estrogen and progesterone are required for both ovulation and female sexual behavior. Although central and peripheral mechanisms of ovulation are well characterized, neural circuit mechanisms underlying the estrus-associated change of the behavior are poorly understood. We have previously shown that progesterone receptor (PR) expressing neurons in the ventromedial hypothalamus (VMH) are essential for female sexual behavior. Targeted ablation of PR+ VMH neurons abolished female sexual behavior while leaving the estrous cycle intact. However, whether PR+ VMH neurons play a role in the estrus-associated change of female sexual behavior or control the behavior independent of estrus is unclear. We examined whether PR+ VMH neurons play a role in the estrus-associated change of female sexual behavior. We find that PR+ VMH neurons significantly strengthen their functional and anatomical connections during estrus with the anteroventral periventricular nucleus. This cyclic increase in connectivity depends on estrogen signaling in PR+ VMH neurons. We further find that these projections are essential for female sexual receptivity during estrus because optogenetic inhibition of these connections abolishes this behavior. These findings demonstrate that periodic remodeling in this behaviorally salient neural pathway plays a critical role in coordinating female sexual behavior with an internal physiological state that coincides with the window of fertility. We will present these and more recent findings to discuss hypothalamic circuit mechanisms that control the timing of female sexual behavior.

Disclosures: S. Inoue: None. **R. Yang:** None. **A. Tantry:** None. **C. Davis:** None. **T. Yang:** None. **J.R. Knoedler:** None. **Y. Wei:** None. **E.L. Adams:** None. **S. Thombare:** None. **S.R. Golf:** None. **R.L. Neve:** None. **M. Tessier-Lavigne:** None. **J.B. Ding:** None. **N.M. Shah:** None.

Poster

P592. Sexual Behavior

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Program #/Poster #: P592.06

Topic: F.02. Behavioral Neuroendocrinology

Support: VIEP-BUAP 2021 to Cuerpo Académico en Neuroendocrinología BUAP-CA-288
CONACyT fellowship No. 662091 to AMB

Title: Evaluation of the olfactory sexual incentive in high- and low-yawning female rats

Authors: *A. MORA-BOLAÑOS[1], C. CORTES[1], J. EGUIBAR[2];
[1]Inst. of Physiol., [2]Res. Office, Vice-rectory of Res. and Postgraduate Studies, Benemérita Univ. Autónoma de Puebla, Puebla, Mexico

Abstract: Sexual attraction in rodents depends on different stimuli such as: smell, audition, and vision, which then activate attention, approach, and finally mating. We selectively bred two sublines from Sprague-Dawley rats that differ in their spontaneous yawning behavior. High-yawning (HY) male rats had a mean of 20 yawns/h and the low-yawning (LY) with just 2 yawns/h. Previously we demonstrated that LY males preferred to mate with HY and SD female rats.The aim of the present study was to evaluate odor discrimination using different bedding soiled from SD, HY and LY sexually experienced male rats. We used an open field arena (OFA) to evaluate odor preference. The bedding material from SD, LY or HY males were randomly assigned to each corner of OFA, and the remaining corner had the same amount of clean wood-shavings. An ovariectomized female rat from each group of rats with an estrous induced by 17-β estradiol followed 44 h later of progesterone was putt gently in the central square of OFA. We measured the bouts and duration of sniffing on each corner, the latency for the first sniffing bout and the return interval to each corner.Our results showed that HY female rats prefer the odors from SD and HY male rats with respect to LY ($P < 0.05$) and they also had the lowest return interval. In conclusion, male pheromones produce a "chemical sexual adornment" that give information at a distance capable of attracting females, and consequently can increase the potential for reproductive success.

Disclosures: A. Mora-Bolaños: None. **C. Cortes:** None. **J. Eguibar:** None.

Poster

P592. Sexual Behavior

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P592.07

Topic: F.02. Behavioral Neuroendocrinology

Title: Impact of a ketogenic diet on sexual behavior in male Sprague-Dawley rats

Authors: C. TZIANABOS, ***L. MARTINEZ**;
Trinity Col., Hartford, CT

Abstract: Ketogenic diets (KDs) are high fat, low carbohydrate, and adequate protein formulations that have shown therapeutic potential for a range of neuropsychiatric disorders, including drug addiction. Previous studies have found that rats administered a KD exhibit reduced cocaine-induced locomotor activity, as well as reduced cocaine- and alcohol-seeking

behaviors. The extent to which KDs impact the expression of behaviors associated with natural rewards such as sex, however, remains to be determined. Given that the limited available evidence from studies utilizing human research participants on KDs has not identified any consistent alterations in sexual function by these diets, we predicted that sexual behavior would remain largely unchanged in male Sprague-Dawley rats administered a KD. To determine if this was indeed the case, sexually-naïve males were assigned to either a KD or a standard chow diet (SD), and then were paired with ovariectomized, hormone-primed females for a series of sexual behavior tests. We found that rats administered a KD exhibited more mounts (across all tests), more intromissions (third and fourth tests) and were less likely to ejaculate (second test) compared to SD males. These data indicate that administration of a KD does impact sexual behavior in male rats, although the high levels of mounts and intromissions seen in these males suggests that motivation to engage in sex remains intact. Future work will determine the extent to which sexual behavior in females may be impacted by KDs.

Disclosures: C. Tzianabos: None. **L. Martinez:** None.

Poster

P592. Sexual Behavior

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Program #/Poster #: P592.08

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH/NINDS F31: 1 F31 NS120801-01
NIH/NIDCR R00-DE026807
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Alfred P. Sloan Foundation
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Columbia University Startup Funds

Title: A skin-to-brain circuit for pleasurable touch and sexual receptivity

Authors: *L. J. MIDDLETON[1], M. D. SCHAFFLER[1], I. K. SUCCI[2], W. FOSTER[2], L. EJOH[1], I. ABDUS-SABOOR[2];
[1]Univ. of Pennsylvania, Philadelphia, PA; [2]Columbia Univ., New York, NY

Abstract: Affiliative social touch not only provides pleasurable hedonic value to relationships but is critical for mental and physical well-being as a means of social buffering. Yet, we lack an understanding of the mechanisms by which we perceive the rewarding valence of social touch, especially how and which peripheral neurons communicate to reward centers in the brain. While C-tactile afferents are thought to convey the affective component of social touch in humans, analogous populations of dorsal root ganglion (DRG) neurons in mice have yet to be implicated

in social behaviors, limiting the power of our experimental models. Here we demonstrate that a small population of mouse DRG neurons marked by developmental expression of Mrgprb4 are both necessary and sufficient for touch-dependent social behaviors and for engaging neural circuits of social reward in the brain. Transdermal optogenetic activation of Mrgprb4-lineage neurons (B4 neurons) in the back skin produces a conditioned place preference, suggesting their focal activation is reinforcing, and thus pleasant. The same focal optogenetic stimulus to the back induces a striking dorsiflexion posture, reminiscent of the lordosis sexual receptivity posture, or dipping their back to crawl underneath cagemates. This posture represents the first acute behavioral response to the optogenetic activation of social touch neurons, positioning social touch in the ranks with itch and pain for yielding a stereotyped, quantifiable motor output upon selective activation. To determine whether B4 neurons are required in natural touch-dependent social postures, we genetically ablated the neurons and examined social behaviors. First, we assessed lordosis. While females without B4 neurons had normal lordotic receptivity on the first pairing with a male, their receptivity fell on subsequent pairings, while controls increased in receptivity. This diminution in receptivity suggests that the B4 neurons are encoding a sensation that positively reinforces female sexual receptivity. To assess whether B4 neurons directly signal to reward centers, we used fiber photometry with the $GRAB_{DA}$ dopamine sensor to measure dopamine release in nucleus accumbens during optogenetic activation of B4 neurons. Further, we measure dopamine release during mating in both intact and B4 neuron-ablated females to see whether B4 neurons are required for reward during sexual encounters. We conclude that B4 neurons are required to mediate touch-dependent social postures, sufficient to replicate them in socially isolated mice, and therefore represent the first neurons of a potential skin-to-brain circuit for social postures and sexual receptivity.

Disclosures: L.J. Middleton: None. **M.D. Schaffler:** None. **I.K. Succi:** None. **W. Foster:** None. **L. Ejoh:** None. **I. Abdus-Saboor:** None.

Poster

P619. Behavioral Effects II

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P619.01

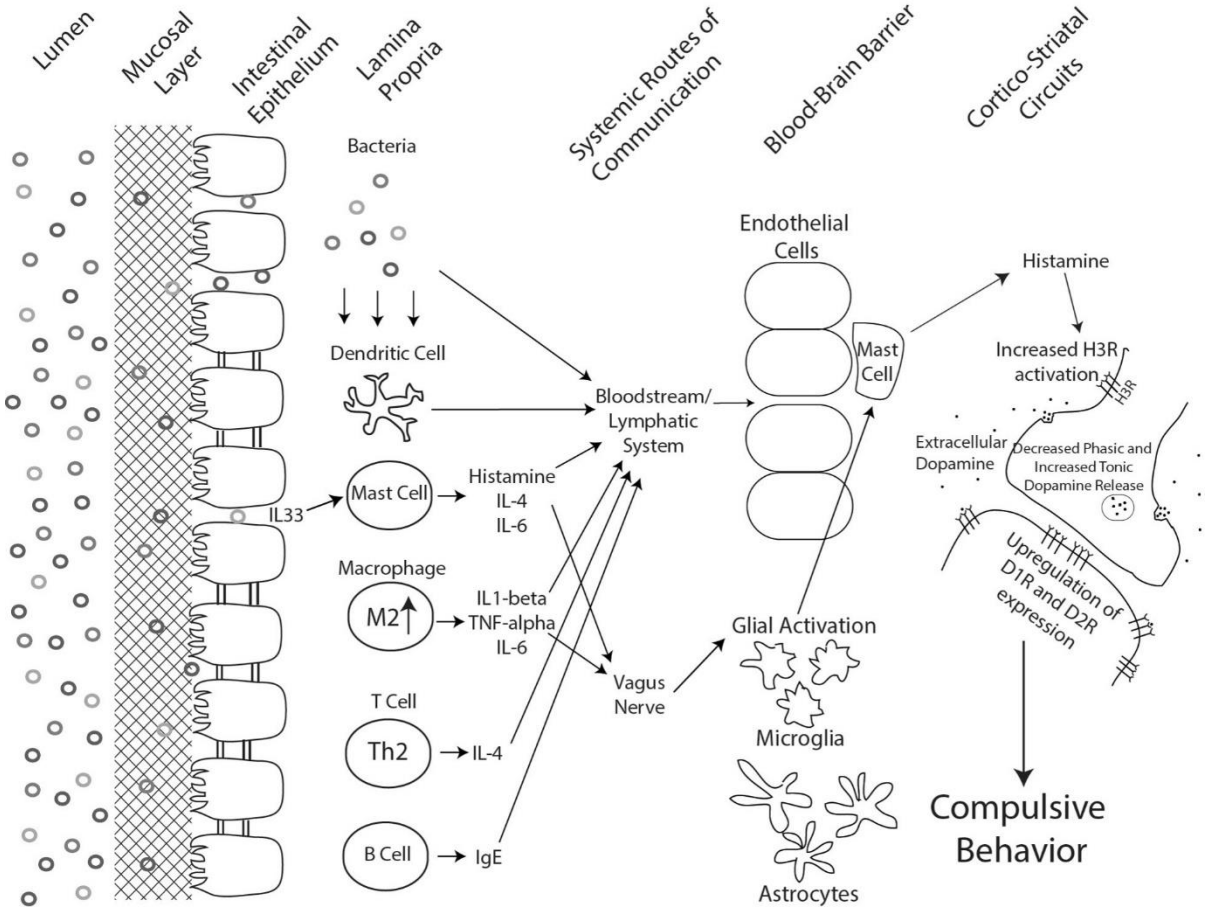
Topic: F.05. Neuroimmunology

Support: R01NS101104-05

Title: Gut Barrier Dysfunction, Type 2 Immunity, and Compulsive Behavior: A Narrative Review of the Literature

Authors: *C. FIELDS;
Yale Univ., New Haven, CT

Abstract: To date, much of the focus of gut-brain axis research has been on gut microbiota regulation of anxiety and stress-related behaviors. Much less attention has been directed to potential connections between gut microbiota and compulsive behavior. The focus of this poster is to review literature suggesting a link between gut barrier dysfunction and compulsive behavior that is mediated through "type 2" rather than "type 1" inflammation. This poster explores connections between compulsive behavior and type 2 inflammation in Tourette syndrome, obsessive-compulsive disorder, autism, addiction, and post-traumatic stress disorder. Potential connections between gut barrier dysfunction, type 2 inflammation, and compulsive behavior are also discussed. I posit a mechanism whereby gut barrier dysfunction-associated type 2 inflammation may drive compulsive behavior through histamine regulation of dopamine neurotransmission. In sum, understanding how gut microbiota affect systemic allergic immune mediators that affect compulsive behavior circuits yields the gut as a readily accessible target for the treatment of compulsive behavior disorders.



Disclosures: C. Fields: None.

Poster

P619. Behavioral Effects II

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P619.02

Topic: F.05. Neuroimmunology

Support: NIMH K01MH117343
WVU Research Office

Title: Alterations in Murine B Lymphocyte Subsets In Response to Stress

Authors: *E. ENGLER-CHIURAZZI, G. TALKINGTON, W. CHASTAIN, C. CASTILLO, B. SWEETEN, B. WHITE, D. CORBIN, J. SIMPKINS, G. BIX, J. TASKER;
Tulane Univ., New Orleans, LA

Abstract: The immune system has emerged as a key regulator of mood, providing novel opportunities for the treatment of debilitating stress disorders such as major depressive disorder (MDD). Several emerging findings highlight key roles for B lymphocytes in modulating the response to stress. For instance, B cell subtype-specific changes were noted in circulation of MDD patients such that while levels of total B lymphocytes were not significantly altered, there were reduced levels of immune-regulating B cells. B lymphocytes have the cellular machinery needed to respond to stress signals and alterations of B lymphocyte numbers in peripheral immune cell compartments have been reported in response to exposure to a number of stress paradigms preclinically. As well, recent evidence of a brain-spleen communication pathway along with our discovery of an age-dependent maladaptive response to the acute swim stress in B lymphocyte deficient mice and our ability to partially ameliorate this phenotype by replacing B cells, further supports this notion. B lymphocytes have been shown to traffic to brain parenchyma in times of neurological challenge (such as stroke). Whether psychosocial stress represents a sufficient neurological challenge to warrant B lymphocyte recruitment to brain is not yet clear. Here we evaluated changes in numbers of splenic and brain B lymphocyte subtypes (total, naïve, antibody-secreting, memory, and regulatory) in response to various acute, chronic and traumatic stress paradigms in young adult C57BL/6 mice. Further, we evaluated age-related changes in splenic B lymphocyte subsets in unstressed mice. We noted stressor-, age-, tissue-, and subtype-specific changes in B lymphocyte numbers. These data contribute support for a growing appreciation for a role for B lymphocytes in modulating the response to stress. Future work will evaluate behavioral and neurobiological responses to stress when B cell numbers are methodically manipulated in vivo.

Disclosures: E. Engler-Chiurazzi: None. **G. Talkington:** None. **W. Chastain:** None. **C. Castillo:** None. **B. Sweeten:** None. **B. White:** None. **D. Corbin:** None. **J. Simpkins:** None. **G. Bix:** None. **J. Tasker:** None.

Poster

P619. Behavioral Effects II

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P619.03

Topic: F.05. Neuroimmunology

Title: Role of Drosophila Stat92E signaling in alcohol addiction-like behavior.

Authors: E. PERIANDRI, ***E. PETRUCCELLI**;
Southern Illinois Univ. Edwardsville, Edwardsville, IL

Abstract: Alcohol use disorder (AUD) is a worldwide health burden; yet our understanding of how repeated alcohol exposure impacts neuromolecular signaling to reinforce maladaptive behaviors like addiction remains incomplete. Similar to human AUD and rodent models, recent transcriptomic analyses in *Drosophila* have demonstrated that memory-associated neurons show changes in the expression of *signal transducer and activator of transcription* (*STAT*), a well-studied innate immune signaling transcription factor, following repeated alcohol exposure. More specifically, flies that make odor cue-associated memories of intoxication, switch from expressing the *Stat92E^{-RI}* isoform, a negative regulator of the JAK/STAT pathway, to *Stat92E^{-RH}* in mushroom body neurons (MB), suggesting that repeated alcohol exposure activates STAT signaling (Petruccelli et al, 2020). Here we explore the necessity of STAT signaling in behavioral sensitization, an enhanced locomotor response to alcohol following pre-exposure. We also examine STAT reporter activity in adult brains and develop a light cue-based associative paradigm in which to further test neuromolecular signaling across addiction-like assays. Ubiquitous *Stat92E* knockdown had a minimal effect on naïve alcohol-induced locomotion, but significantly increased behavioral sensitization in flies previously exposed to alcohol. STAT reporter activity was not altered upon alcohol pre-exposure, but showed restrictive expression to the α/β MB lobes upon pan-neuronal overexpression of constitutive active STAT. Lastly, we provide preliminary evidence that blue light can be used as an associative cue for intoxication experience, which will aid in further evaluating the role of STAT signaling in conditioned preference behavior. Together this work begins to resolve the complex spatiotemporal neuroimmune signals underlying addiction-associated behaviors.

Disclosures: E. Periandri: None. **E. Petruccelli:** None.

Poster

P619. Behavioral Effects II

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P619.04

Topic: F.05. Neuroimmunology

Support: Neurolign USA LLC

Title: Oculomotor, vestibular, reaction time and cognitive (OVRT-C) tests in recovered COVID-19 patients

Authors: *K. M. KELLY[1], R. ANGHINAH[2], A. KULLMANN[5], A. S. SYNOWIEC[1], L. GIBSON[6], L. MANFRINATI[7], A. DE ARAUJO[3], R. SPERA[4], S. BRUCKI[8], A. BRAVERMAN[9], A. KIDERMAN[5];
[1]Neurol., Allegheny Hlth. Network, Pittsburgh, PA; [2]Ambulatorio de Reabilitacao Cognitiva (ARCO) and Med. Dept. of Athletes Union of Sao Paulo, [3]ARCO, Clin. Hosp. of Med. Sch. of Univ. of São Paulo, Sao Paulo, Brazil; [4]Clin. Hosp. of Med. Sch. of Univ. of São Paulo, São Paulo, Brazil; [5]Neurolign USA LLC, Pittsburgh, PA; [6]Neurolign Technologies Inc., Toronto, ON, Canada; [7]Ambulatorio de Reabilitacao Cognitiva (ARCO) and Med. Dept. of Athletes Union of Sao Paulo, Clin. Hosp. of Med. Sch. of Univ. of Sao Paulo, Sao Paulo, Brazil; [8]Clin. Hosp. of Med. Sch. of Univ. of São Paulo, São Paulo, Brazil; [9]The Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: More than 30% of patients infected by SARS-CoV-2 (COVID-19) experience long-lasting neurological symptoms (e.g., headache, dizziness, mental status changes), which could have long term consequences. However, the evaluation of COVID-19-related neurological deficits is often subjective and underlying neural substrates are not understood. Eye movements have been traditionally used as a non-invasive readout of many brain dysfunctions, and thus deficiencies in oculomotor behavior can provide insights into the neural substrates impacted by COVID-19 infection. We used a highly sensitive and precise video-based eye-tracking technology to evaluate OVRT-C deficits in patients who recovered from acute COVID-19 infection. Adult men and women were recruited at the Allegheny General Hospital (N=13) and University of São Paulo School of Medicine, Brazil (N=13), under approved IRB protocols. Subjects (N=26) were tested at various times following recovery with a battery of OVRT-C tests delivered by a portable videonystagmograph (Dx100, Neurolign Technology, Inc.), and reported neurological symptoms using the Neurobehavioral Symptom Inventory (NSI). Patients' OVRT-C metrics were compared with FDA-cleared normative data of 466 males and females 18-45 years old. Various OVRT-C deficits were present in a percentage of patients. These included deficits in: a) oculomotor tests such as saccade (30% and 33% of patients for horizontal and vertical, respectively) and vergence (33%); b) vestibular tests such as optokinetic nystagmus (OKN, 27.3%); c) reaction time tests (15% and 12% for visual and auditory, respectively); and d) cognitive tests such as antisaccade (36%) and predictive saccade (27.3%). Preliminary evaluation of the relationship between NSI data and OVRT-C deficits showed that dizziness and balance symptoms were strongly correlated with gaze tests ($p=0.03$), fatigue with antisaccade errors ($p=0.04$), and irritability with gain in OKN tests ($p<0.05$). Logistic regression univariate model identified OVRT-C metrics that discriminated between controls and patients. These included gain in the OKN test (60 deg/s; AUC 0.86), percentage of predicted saccades (AUC 0.82), and percentage of prosaccade errors in the antisaccade test (AUC 0.79). These preliminary results suggest that videonystagmography can successfully identify OVRT-C deficits. Early identification of neural deficits caused by COVID-19 may help to better understand the neural substrates impacted by COVID-19 infection, aid in its management, and ideally prevent its long term consequences.

Disclosures: K.M. Kelly: 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.; Neurolign USA LLC. **R. Anghinah:** None. **A. Kullmann:** None. **A.S. Synowiec:** None. **L. Gibson:** A. Employment/Salary (full or part-time):; Neurolign Technologies Inc.. **L. Manfrinati:** None. **A. de Araujo:** None. **R. Spera:** None. **S. Brucki:** None. **A. Braverman:** 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.; Neurolign Technologies Inc. **A. Kiderman:** A. Employment/Salary (full or part-time):; Neurolign USA LLC.

Poster

P619. Behavioral Effects II

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P619.05

Topic: F.05. Neuroimmunology

Title: Exploring the effects of adolescent amphetamine sensitization and trait anxiety on NF-kB and TNF-a protein expression in a male Long-Evans rat model

Authors: *C. CALHOUN[1], S. ROY[1], J. SEVERINO-PEREZ[1], V. LEWIS[1], B. PLOTKIN[1], T. DONALDSON[2];
[1]Univ. of Massachusetts, Boston, MA; [2]Univ. of Massachusetts Boston, Boston, MA

Abstract: Mammalian adolescence is a developmental period that confers increases in impulsive and reward-seeking behavior paralleled by heightened sensitivity to affect and stress. Both human and rodent literature highlight the dynamic remodeling of the mesocorticolimbic pathway in this behavioral shift and in the vulnerability to drug abuse. Advances in addiction neuroscience center the toll-like receptor 4 (TLR4) system within substance abuse paradigms and emphasize the role of downstream transcription factors and cytokines in the neuroplasticity associated with progressive stages of drug addiction. The present study aims to explore the expression of TLR4-downstream substrates, Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-kB) and Tumor Necrosis Factor alpha (TNF-α) protein expression, after exposure to an AMPH sensitization protocol. Thirty adolescent male Long-Evans rats were phenotyped on the elevated-plus maze (EPM) for extreme high (HAn) and low (LAn) anxiety-like behavior. Following EPM screening, animals went through a 4-day repeated AMPH protocol, where animals were injected with AMPH (4.0 mg/kg IP) or vehicle (0.9% NaCl) and tested in the open field chamber for locomotor activity. HAn adolescents exhibited the most hyperactivity to the acute AMPH injection across Days 2-4 and HAn animals displayed the greatest locomotor response to a low challenge dose of AMPH (1.0 mg/kg IP) after a 7-day

withdrawal period. Histological analyses indicated differential expression of TLR4 protein in the basolateral amygdala, with higher expression in HAn rats. These results suggests that extreme trait anxiety-like phenotypes can differentiate responses to repeated AMPH exposure and map onto differential proinflammatory responses in an adolescent rat model.

Disclosures: C. Calhoun: None. **S. Roy:** None. **J. Severino-Perez:** None. **V. Lewis:** None. **B. Plotkin:** None. **T. Donaldson:** None.

Poster

P619. Behavioral Effects II

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P619.06

Topic: F.05. Neuroimmunology

Support: NARSAD 2019 Young Investigator Grant
NICHD R01 HD072968

Title: Developmental role of mast cells in adult mouse emotional behaviors and neuroinflammatory responses.

Authors: *N. DUQUE-WILCKENS[1], A. ROBISON[2], A. J. MOESER[1];
[2]Neurosci., [1]Michigan State Univ., East Lansing, MI

Abstract: Mast cells (MCs) are bone marrow derived innate immune cells distributed throughout nearly all tissues, including the brain. MCs can store and release a wide spectrum of preformed and newly synthesized biologically active mediators, including neurotransmitters, affecting the function of multiple systems. Most of the knowledge about MC function *in vivo* has been derived from studies on constitutive, KIT-dependent MC deficiency models, which do not allow for the differentiation developmental vs. acute effects. Here we crossed the Mcpt5-Cre mouse line with the Cre-dependent B6-iDTR (Jax®) mice to render MCs susceptible to ablation following diphtheria toxin (DTX) administration with the objective of determining the developmental role of MCs in adult behavior and brain neuroinflammatory responses. We injected the F1 litters with 30ng/g of DTX every 7 days starting at different developmental stages until adulthood (8 weeks old). Adult animals were tested in the elevated plus maze and sucrose preference tests to assess behavior, and then injected intraperitoneally with 1mg/kg of lipopolysaccharide (LPS) to measure acute thermoregulatory and inflammatory responses to an immune challenge. Compared to MCPT5-Cre-;iDTR (controls), MCPT5-Cre+;iDTR (ablated), show increased time spent in the open vs. closed arms in the elevated plus maze when DTX injections start between PN1 and PN28, but not when MCs were ablated in adulthood, suggesting an organizational role of MCs during adolescence on neural circuits modulating anxiety. Ongoing measures include quantification of circulating cytokines and neuroinflammatory responses to LPS. The results of

these studies will provide new knowledge about the critical periods in which MCs exert developmental effects on neuroimmune systems regulating anxiety behavior.

Disclosures: N. Duque-Wilckens: None. **A. Robison:** None. **A.J. Moeser:** None.

Poster

P619. Behavioral Effects II

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Program #/Poster #: P619.07

Topic: F.05. Neuroimmunology

Support: NIH-NIGMS 5R25GM082406 (PHSU RISE Graduate Training Program)
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NIH-NIGMS R15MH116345-01
NIH-NIGMS R25GM096955 (UPR-Ponce PRISE Program)

Title: Localized infusion of C20 ceramide triggers reversible short-term behavioral changes in female and male rats

Authors: *L. SAMBOLIN-ESCOBALES[1], A. FELICIANO-QUIÑONES[2], C. SUAREZ-GOMEZ[2], L. TIRADO-CASTRO[2], W. FONSECA-FERRER[3], K. SANTOS-AVILES[2], M. COLON-ROMERO[2], A. HERNANDEZ-LOPEZ[2], J. PORTER[2];
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Abstract: Previous data showed that rats with depressive-like behavior showed increased concentration of C20 ceramides in serum. Moreover, depressive-like behavior has been associated with increased microglia and the production of inflammatory mediators. However, it is unclear whether increasing C20 ceramides in the brain is sufficient to induce depressive-like behaviors and activate microglia and astrocytes which could induce localized production of inflammatory mediators. We hypothesized that infusion of C20 ceramides into the ventral hippocampus (VH) of rats would activate microglia and astrocytes to induce depressive-like symptoms. To test this hypothesis, adult male and female rats received 7 single infusions of C20 ceramides or vehicle into the VH every 48 hours to assess short-term behavioral changes. Twenty-four hours after each C20 ceramide infusion, a sucrose preference test (SPT) was used to determine changes in anhedonia-like behavior. Animals injected with C20 ceramides showed less preference for sucrose suggesting increased anhedonia-like behavior. Five days after the last infusion of C20, a sucrose grooming test (SGT) and forced swim test (FST) were performed to examine long-term changes in anhedonia and depressive-like behaviors. All groups showed similar behavior in the SGT and the FST, suggesting that behavioral changes induced by the C20 ceramide infusions was reversible. Animals were sacrificed after the final FST, and immunofluorescence staining was performed to determine the expression of Iba-1 and GFAP to

assess microglia and astrocyte activation. The infusion of C20 ceramides increased the number of microglial cells in the VH, but no changes were noted in the expression of astrocytes. In conclusion, our data suggest that the localized presence of C20 ceramides in the VH of rats is sufficient to cause short-term reversible behavioral changes and activate microglia.

Disclosures: L. Sambolin-Escobales: None. **A. Feliciano-Quiñones:** None. **C. Suarez-Gomez:** None. **L. Tirado-Castro:** None. **W. Fonseca-Ferrer:** None. **K. Santos-Aviles:** None. **M. Colon-Romero:** None. **A. Hernandez-Lopez:** None. **J. Porter:** None.

Poster

P619. Behavioral Effects II

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Program #/Poster #: P619.08

Topic: F.05. Neuroimmunology

Support: NIH/NIGMS Grant P20GM103442
Host-Pathogen CoBRE Pilot Grant P20GM113123
University of North Dakota SMHS Department of Biomedical Sciences

Title: Repeated allergen exposure triggers behavioral and neuroinflammatory changes in a mouse model of asymptomatic cow's milk allergy

Authors: *D. GERMUNDSON[1], A. BRISHTI[2], N. A. SMITH[3], K. NAGAMOTO-COMBS[2]; [1]Pathology, [2]Biomed. Sci., Univ. of North Dakota SMHS, Grand Forks, ND; [3]Pathology, Univ. of North Dakota Sch. of Med. and Hlth. Sci., Grand Forks, ND

Abstract: Growing evidence suggests that neuropsychiatric disorders are often associated with food allergies in both children and adults. Using a mouse model of non-anaphylactic cow's milk allergy, we previously demonstrated that allergic sensitization of male C57BL/6J mice produced aberrant behaviors and brain pathology without inducing severe reactions upon allergen challenge. While these results suggested the causal role of food allergy in behavioral changes after acute allergen exposure, the effects of prolonged allergen exposure on the brain are unknown. Since allergic individuals with mild symptoms can consume offending foods without life-threatening consequences, they may be subjected to repeated allergen challenges. We hypothesized that repeated allergen consumption by mildly allergic individuals could promote neuroinflammation and behavioral changes via chronic systemic inflammation. To test our hypothesis, we orally sensitized 4-week-old male C57BL/6J mice to a bovine whey allergen, β-lactoglobulin (BLG), and placed them on a diet containing 0.3% whey proteins for 2 weeks to simulate repeated allergen exposure. The tail suspension test showed increased depression-like behavior in BLG-sensitized mice on the whey diet compared to sham mice. Despite the absence of obvious allergic symptoms in the BLG-sensitized mice, serum histamine and allergen-specific

IgE were elevated. Plasma levels of IL-6, IL-17, and IFNγ were also elevated in BLG-sensitized mice, indicating that peripheral inflammation was maintained by continued allergen consumption. In the brain, we observed increased IgG immunoreactivity in the parenchyma and GFAP-immunoreactive hypertrophic astrocytes in perivascular regions, suggestive of immunoglobulin extravasation from 'leaky' blood-brain barrier (BBB) and neuroinflammation, respectively. Greater numbers of mast cells and elevated histamine levels were also found in the brains of BLG-sensitized mice, likely contributing to the observed changes in BBB permeability. Importantly, we found a decrease in the density of myelination in the motor and somatosensory cortices in BLG-sensitized mouse brains. Our findings indicate that repeated allergen exposure significantly altered behavior of BLG-sensitized mice, likely through neuroinflammation and demyelination. In certain individuals, neuropsychiatric symptoms may be triggered or exacerbated by subclinical immune responses that ultimately affect brain function when prolonged. Thus, food allergy testing and allergen avoidance may prove useful as strategies for treating and preventing neuroinflammatory and neuropsychiatric disorders.

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Poster

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Program #/Poster #: P619.09

Topic: F.05. Neuroimmunology

Support: NARSAD
NICHD R01 HD072968

Title: Mast cell-serotonin interactions as a potential mechanism underlying anti-anxiety effects of mast cells

Authors: *R. TEIS[1], N. MARADIAGA[2], A. J. MOESER[2], A. ROBISON[3], N. DUQUE-WILCKENS[4];
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Abstract: Mast cells (MCs) are known for their involvement in peripheral diseases such as allergy, but they are also present in the brain where they can modulate behavior. MC deficient (Wsh/Wsh) mice show a phenotype consistent with anxiety/anhedonia: compared to wild type mice, Wsh/Wsh spend less time in the open vs. closed arms of the elevated plus maze test as well as reduced sucrose preference. We show that these phenotypes can be normalized by central reconstitution of Wsh/Wsh mice with wild type bone marrow derived mast cells (BMMC),

suggesting that brain MCs exert mood-stabilizing effects. MCs could reduce anxiety by modulating the brain's serotonergic neural network, as MCs can synthesize, store, and quickly release a variety of molecules that directly affect neuronal activity, including histamine, cytokines like IL-6, and serotonin itself. Indeed, both pharmacological activation and conditional knock-out of MCs affect serotonin content in various brain nuclei, although it is not clear whether this contribution is a direct result of MC serotonin release or MC modulation of neural serotonergic circuits. Here we used immunohistochemical quantitation of serotonin and c-Fos to characterize the number, distribution, and activation levels of dorsal raphe serotonin neurons in wild type, Wsh/Wsh, and Wsh/Wsh mice with central or peripheral BMMC reconstitution after acute exposure to mild stress. Preliminary data demonstrate that, compared to wild type, Wsh/Wsh mice show less serotonin+ cells in anterior dorsal raphe and that this is partially reversed by both peripheral and central reconstitution with BMMCs, suggesting that circulating MC products could modulate central serotonin expression. Ongoing studies are assessing dorsal raphe expression of mRNA encoding for serotonin transporter as well as tryptophan hydroxylase 1 and 2 in Wsh/Wsh and wild type mice. Our findings indicate the MCs within and outside the brain can regulate serotonergic neurons, a potential mechanism by which these innate immune cells may affect mood and anxiety.

Disclosures: R. Teis: None. **N. Maradiaga:** None. **A.J. Moeser:** None. **A. Robison:** None. **N. Duque-Wilckens:** None.

Poster

P685. Hormones and Behavior: Motivation and Social Behavior

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P685.01

Topic: F.02. Behavioral Neuroendocrinology

Support: MH122622

Title: Neuronal activation induced by social communication in syrian hamsters

Authors: *J. W. VANDER VELDEN, N. POURSHIRAVI, B. W. HORNE, J. H. TAYLOR, K. L. HUHMAN, H. E. ALBERS;
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Abstract: Social communication plays a critical role in nearly all social interactions. Many mammalian species communicate with chemosensation, and for solitary species chemosensation can be critical for social interactions. Chemosensation also allows some species to communicate indirectly because chemical signals such as odor marks can be used to mark territory and to indicate reproductive or social status. Syrian hamsters engage in a scent marking behavior called flank marking during social interactions and in response to the odors of other hamsters. In the

present study, we investigated whether odor-induced flank marking results in the activation of neurons in hypothalamic regions, such as the anterior hypothalamus (AH), paraventricular nucleus (PVN), and supraoptic nucleus (SON), that are important for social behavior in hamsters. Male and female hamsters (n=8 each) were exposed to either a clean cage (control) or a cage that was previously marked by a conspecific. The number of flank marks observed in hamsters exposed to conspecific odors (26.27±3.35; Mean±SEM) was significantly greater than in controls (13.31±3.35) (p=0.01). Ninety minutes after behavioral testing, brains were taken and processed for immunohistochemical localization of cFos. In hamsters exposed to conspecific odors, expression of cFos in the AH (p=0.044) and the PVN (p=0.036) was significantly greater than in controls. We also observed a trend in the SON (p=0.073) for less neuronal activation in odor-exposed hamsters compared to controls. These data support the hypothesis that activation of neuronal activity in the AH and PVN along with inhibition of neuronal activity in the SON may contribute to the regulation of odor-stimulated flank marking. Supported by MH122622.

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Poster

P685. Hormones and Behavior: Motivation and Social Behavior

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Program #/Poster #: P685.02

Topic: F.02. Behavioral Neuroendocrinology

Support: P50MH100023
R01MH112788
P51OD011132

Title: Melanotan II increases oxytocin-dependent neuronal activity in social brain areas

Authors: *C. L. FORD, IV[1], A. A. MCDONOUGH[1], L. J. YOUNG[2];
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Abstract: Autism spectrum disorders (ASD) are characterized by deficits in social cognition, yet there are no pharmaceuticals approved to treat these deficits and the efficacy of behavioral therapies are limited by the social nature of communication with a therapist. The neuropeptide oxytocin increases the salience of social stimuli and induces pro-social behavioral effects in animals and humans. Activation of the oxytocin system is therefore a potential therapeutic target for ASD. However, most studies testing intranasal administration of oxytocin have yielded small effect sizes due to limited brain penetration and short half-life in peripheral circulation. Moreover, no studies have attempted to enhance behavioral therapy with oxytocin. We propose that pharmacologically activating the endogenous oxytocin system immediately prior to

behavioral therapy may offer therapeutic benefits. Melanotan II (MTII), a melanocortin receptor agonist, induces the release of endogenous oxytocin in the hypothalamus. Our previous studies in socially monogamous prairie voles demonstrated that 10 mg/kg MTII injected IP accelerates pair bond formation in an oxytocin-dependent manner and potentiates the release of oxytocin in the nucleus accumbens. Here, we investigate the neural effects of centrally administered MTII. First, we injected MTII ICV at different doses (0, 0.001, 0.01, 0.1, 1.0 nmol) and assessed behavioral stereotypies in a dose-response curve. Results indicated 0.1 nmol was the optimal dose for future studies. We next infused 0.1 nmol MTII, 0.1 nmol MTII combined with an oxytocin antagonist, or aCSF ICV and exposed voles to either a novel object or a social experience with a novel conspecific. To assess the neuronal activity induced by MTII, we quantified expression of the immediate early gene protein c-Fos as a proxy for neuronal activity in brain regions involved in social cognition. We found MTII increased c-Fos expression in the prelimbic cortex ($p<.05$) in both social and nonsocial contexts. This effect in the social context was blocked by coadministration of an oxytocin antagonist. Additional brain regions are being analyzed, but preliminary data suggest MTII may evoke the release of endogenous oxytocin and thereby activate brain networks involved in social cognition. Therefore, administering MTII immediately prior to behavioral therapy may increase social information processing by evoking endogenous oxytocin release and thus has potential to increase the efficacy of therapy in treating the social deficits of ASD.

Disclosures: C.L. Ford: None. **A.A. McDonough:** None. **L.J. Young:** None.

Poster

P685. Hormones and Behavior: Motivation and Social Behavior

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Topic: F.02. Behavioral Neuroendocrinology

Support: NIH K01MH121575
NIH T32 MH093315
NIH CTSA UL1TR002489
Foundation of Hope for Research and Treatment of Mental Illness

Title: Mood sensitivity to sex hormone flux predicts oscillatory correlates of fronto-limbic affective processing

Authors: *E. ANDERSEN, K. BARESICH, S. GIRDLER;
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Abstract: Background: The pubertal transition (PT, peripuberty) is characterized by pronounced sex hormone fluctuation, dramatic neuromaturation and marks a divergence between the sexes in

risk of depression in girls. A heterogeneous pattern of fronto-limbic neural development, with more rapid growth of limbic affective regions overshadowing a delayed maturation of frontal networks critical for top-down emotion regulation, predisposes adolescents to dysfunctional emotional reactivity. The functional coupling of distributed frontal and limbic neural networks relies on synchronous neural oscillations in theta (4-8 Hz) and beta (12-30 Hz) frequencies, which can be directly measured using EEG. Sex hormones, including testosterone (T) and estrone (E1), exert significant modulatory effects on frontal and limbic affective systems and have been associated with depression symptoms; however, the neurophysiological mechanisms linking sex hormones with affective state change in the PT have yet to be defined. The objective of the present study was to determine whether mood sensitivity to weekly changes in T and E1 differentially impacted frontal theta and parietal beta in response to emotional stimuli, indexing dysregulated fronto-limbic affective processing.
Method: 44 peripubertal females (ages 11-14, within 1 year of menarche) provided 8-weekly salivary hormone (T, E1) samples (pg/mL) and mood assessments, and completed an emotional go-no/go task during EEG recording. Event-related theta (4-8 Hz) and beta (12-30 Hz) power was extracted from frontal (Fz) and parietal (Pz) midline electrodes, respectively, in response to emotional facial stimuli. Within-person correlations between weekly change in hormone (T, E1) and corresponding mood ratings were performed to determine individual differences in mood sensitivity to hormone flux.
Results: Peripubertal females who were mood-sensitive to T exhibited enhanced frontal theta oscillatory activity ($F(1,42=11.06$, $p=.002$, $\eta_p^2=0.21$), whereas, participants mood sensitive to E1 demonstrated increased parietal beta activity in response to negative emotional stimuli ($F(1,42=6.51$, $p=.014$, $\eta_p^2=0.13$), reflecting exaggerated attention and emotional reactivity.
Conclusion: For the first time, results suggest that susceptibility to T and E1 fluctuations and affective state change may rely on unique neurophysiological mechanisms, with E1 targeting beta-dependent localized limbic networks, and T implicated in frontal regulation of behavior and emotion. Thus, aberrant frontal theta and parietal beta activity may link sensitivity to hormone change and mood symptoms during this pivotal developmental window.

Disclosures: E. Andersen: None. **K. Baresich:** None. **S. Girdler:** None.

Poster

P685. Hormones and Behavior: Motivation and Social Behavior

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P685.04

Topic: G.02. Reward and Appetitive Learning and Memory

Title: Impact of estrous cycle on reward-based probabilistic behavior in female rats

Authors: A. TEJADA, J. BEZENAH, B. VAZQUEZ, D. GARCIA, M. ESPINOSA, ***L. R. AMODEO**;
Psychology, CSU San Bernardino, San Bernardino, CA

Abstract: Emerging evidence has shown that sex differences are present in probabilistic decision-making when using a rat version of the Iowa gambling task or a delay discounting task. While much of the research investigating probabilistic decision-making, or risk-taking behavior, has been conducted in male subjects, the evidence that exists regarding an influence of estrous cycle in decision making is mixed. Changes in estrus cycle can be associated with behavioral alterations, particularly approach, anxiety and cognitive behaviors. Thus, we propose to investigate whether probabilistic choice behavior shifts depending on stage of estrous cycle. Parous and nulliparous female Long-Evans rats were tested for risk-preference using a 2-choice probabilistic gambling paradigm. For this task, rats were given an option between a 'large-risky' choice (3 sugar pellets delivered on a probabilistic VR schedule) and 'small-certain' choice (1 sugar pellet delivered on a FR1 schedule). Once rats learned to discriminate reward magnitudes, one level of risk (16%, 33%, 67%) was randomly assigned to the 'large-risky' choice. Each rat was randomly presented with the probabilities twice for a total of 6 sessions. Phases of estrous cycle (diestrus, proestrus, or estrus) were determined cytologically through vaginal lavage taken 30 minutes after completing each behavioral session. Our preliminary findings indicate that female rats discriminate between varying probabilistic schedules with estrous cycle not impacting overall risk-preference.

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Poster

P685. Hormones and Behavior: Motivation and Social Behavior

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Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P685.05

Topic: F.02. Behavioral Neuroendocrinology

Support: Iowa Osteopathic Education and Research Foundation

Title: Estrogen regulates voluntary running behavior

Authors: *V. MATHIS, L. J. POINTS, B. POPE, L. WINTER, S. CLAYTON, L.-L. YUAN;
Des Moines Univ., Des Moines, IA

Abstract: Despite the myriad social and health benefits of exercise, humans display heterogeneous levels of participation. Significant progress has been made in identifying molecular events, systems, and mechanisms that support exercise's beneficial effects, but it is not

clear yet what regulates exercise behavior itself or serves to maintain prolonged chronic exercise behavior. The human heterogeneity in voluntary exercise can be recapitulated in a rodent model of wheel running, a behavior with high rewarding properties. While rats given continuous access to running wheels all began with low running activity, a 3-week training program dramatically increased running activity and revealed a wide range of individual differences. In addition, we have also identified intriguing sex differences. Compared to age-matched males, female rats exhibited significantly higher levels of average daily running; individual females also exhibited a repetitive peak-valley pattern of running activity, with peaks coinciding with the proestrus stage (highest estrogen level) in the rat estrous cycle. Ovariectomy (OVX) not only drastically lowered their running activity, but also completely eliminated the running peaks. Furthermore, baseline estrogen replacement via osmotic mini-pumps in an OVX background restored running activity to pre-OVX levels, and acute estradiol injections were able to mimic running peaks. Collectively, our results suggest estrogen regulates running activity which offers a unique opportunity to examine the mechanisms responsible for driving exercise behaviors. Further studies identifying molecular mechanisms that mediate estrogen's effects are currently underway.

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Poster

P685. Hormones and Behavior: Motivation and Social Behavior

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Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P685.06

Topic: F.02. Behavioral Neuroendocrinology

Title: Brain derived neurotrophic factor changes in response to different sucrose binge drinking concentrations in adolescent male rats

Authors: *Z. SIMSEK[1], A. TORRES[1], J. SANTOS[3], C. TYLER[2], O. DARBANDI[2], K. HOANG[4], K. S. CURTIS[5], D. B. VAZQUEZ SANROMAN[1];
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Abstract: Binge-like consumption of sucrose causes an escalation in intake over time similar to self-administration of drugs of abuse, suggesting that sugar binge drinking (SBD) may lead to a sensitization of the reward system. High levels of sugar intake trigger release of brain-derived neurotrophic factor (BDNF) in the brain. Nevertheless, it is not clear how SBD translates into changes in BDNF levels in brain reward areas. Therefore, the specific aim of this study was to determine the effect of SBD on proBDNF and MatureBDNF (mBDNF) in the adolescent PFC and Nacc. **Methods**: Male rats [Postnatal day (PND) 26] were randomly assigned to the SBD

group (5% or 15%) or the control group, water and chaw were available ad libitum. SBD sessions consisted of intermittent access to either 5% or 15% sucrose concentrations (120 minutes/day for five consecutive days), with having water always available. After the last SBD session, rats were sacrificed, and brains were extracted and prepared for immunoassay analysis (ELISA) for identifying pro- and mBDNF levels. **Results:** adolescent rats showed a 10-fold increase in proBDNF levels compared to adults. Also, juvenile rats displayed more pronounce SBD behavior at 5% compared to 15 % sucrose concentration. Furthermore, SBD at 5% but not at 15% increased proBDNF level in the Nacc. Surprisingly, SBD at both 5 and 15% concentration increased levels of proBDNF in the PFC. Thus, adolescent binge sucrose drinking may sensitize the reward system by selective alterations in BDNF levels.

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Poster

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Program #/Poster #: P685.07

Topic: F.02. Behavioral Neuroendocrinology

Support: R01 AA027645
T32 DA03980

Title: The reciprocal relationship between estrogen and binge alcohol drinking

Authors: *L. J. ZALLAR, J. K. RIVERA, K. E. PLEIL;
Pharmacol., Cornell University: Weill Cornell Med. Col., New York, NY

Abstract: Pathological alcohol use is a widespread public health concern that incurs significant societal and personal costs. Estrogen (17β-estradiol; E2) is a sex steroid hormone synthesized in the brain and periphery through the process of aromatization, in which the rate-limiting enzyme aromatase converts the steroid hormone testosterone to E2. In females, E2 produced in the ovaries fluctuates across the estrous/menstrual cycle to promote pro-survival and reproductive behaviors including reward/aversion sensitivity and learning, and anxiolysis. As such, E2 may promote early alcohol drinking behavior in females via modulation of reward and stress-sensitive neural circuits, as well as behavioral and pharmacological interactions with alcohol. Our preliminary data show that female mice with high ovarian E2 status binge drink more and display decreased anxiety compared to when they are in a low E2 state. Further, direct E2 administration to the BNST recapitulates these behavioral effects and increases glutamate release onto $BNST^{CRF}$ neurons, which we have previously shown drive drinking behavior, suggesting that $BNST^{CRF}$ neurons may be a target for E2's behavioral effects on binge drinking. E2 has been

shown to act as a neurotransmitter in the brain, including in the BNST, allowing it to rapidly regulate many behaviors via binding to E2 receptors. In addition, chronic alcohol exposure may be able to promote aromatization through a currently unknown mechanism. We have found that binge alcohol consumption is sensitive to pharmacological aromatase inhibition following 3 weeks of exposure in males and 10 weeks in females. Thus, there may be a feedforward relationship between E2 and alcohol consumption in both sexes, such that alcohol exposure recruits aromatase leading to increased synthesis of E2, which in turn signals in the BNST to drive further alcohol consumption. Ongoing experiments are utilizing slice electrophysiology, western blot, and other converging techniques to characterize the sources and mechanisms by which alcohol and E2 interact.

Disclosures: L.J. Zallar: None. **J.K. Rivera:** None. **K.E. Pleil:** None.

Poster

P685. Hormones and Behavior: Motivation and Social Behavior

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Program #/Poster #: P685.08

Topic: F.02. Behavioral Neuroendocrinology

Support: NIDDK NIH R01

Title: Conditional knock-out of estrogen receptor beta in corticotrophin releasing hormone neurons alters feeding and anxiety-like behavior in a sex-specific manner

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Abstract: Single-minded homolog 1 (Sim1) is a transcription factor that is essential for development of the paraventricular nucleus (PVN) of the hypothalamus. Global haploinsufficiency of Sim1 in both humans and mice is associated with hyperphagic obesity. Conditional knock-out of estrogen receptor beta (ERB) in Sim1 ($Sim1^{Cre}$/ $ERB^{fl/fl}$) containing neurons in mouse PVN produced increases in body mass in both males and females on a regular chow diet. The precise subpopulation of Sim1 neurons which produces these body mass changes is presently unknown, but Sim1 neurons in the PVN overlap with oxytocin, corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP)-containing neurons. In the present study, $ERB^{fl/fl}$ mice were cross-bred to CRH^{Cre} mice to produce a novel transgenic mouse with ERB knocked out of CRH neurons (CRH^{Cre}/ $ERB^{fl/fl}$). Changes in both feeding behavior and anxiety-like behavioral assays were observed in a sex-specific manner. Female CRH^{Cre}/ $ERB^{fl/fl}$ mice exhibited an increase in food intake over time compared to CRH^{Cre} and $ERB^{fl/fl}$ female controls ($p<0.05$). Male CRH^{Cre}/ $ERB^{fl/fl}$ mice exhibited increases in both total time spent

moving and total distance traveled in the elevated plus maze assay compared to CRH^{Cre} and $ERB^{fl/fl}$ male controls ($p<0.05$). These findings suggest a potential role for neurons containing both ERB and CRH in mediating anxiety-like behavior in males and feeding behavior in females.

Disclosures: R. Daniels: None. **J.A. Sheng:** None. **A.M. Miller:** None. **R.J. Handa:** None.

Poster

P685. Hormones and Behavior: Motivation and Social Behavior

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P685.09

Topic: F.02. Behavioral Neuroendocrinology

Support: Herman Dana Foundation.

Title: Prospective cohort study of neuroendocrine reactivity and prognostic outcome among adolescents with restrictive Anorexia Nervosa.

Authors: *T. GOLTSER[1], R. GIESSER[2], A. SHALEV[2], R. MASARWA[2], Y. MARTIN[2], A. MELTZER[2], R. RATZON[3], D. PEVZNER[3], L. CANNETI[4], E. GALILI-WEISSTUB[3], R. SEGMAN[5];
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Abstract: Prospective cohort study of neuroendocrine reactivity and prognostic outcome among adolescents with restrictive Anorexia Nervosa.
Goltser Dubner T[1,2],Giesser R[1], Shalev A[1], Masarwa R[1], Ratzon R[1], Martin Y[1], Meltzer A[1],Masarwa R[1], Pevzner D[1,2]; Canetti L[2], Galili-Weisstub E[1], Segman R[2].
Abstract:
Background: The aim of the current study is to prospectively characterize neuroendocrine reactivity among adolescent AN patients and correlate them with long term prognostic outcomes.**Methods:** AN patients referred to the ambulatory clinic, day care facility and in patient unit of the Herman Dana Child Psychiatry Center were assessed using a structured clinical interview with repeated psychological and physical assessments. Hormonal assays were sampled at baseline and following treatment, correlated with treatment response measures, and compared with healthy age matched adolescents. **Results:** AN patients demonstrate a significant prospective increase in BMI compared with baseline, and improvement in pathological eating attitudes at follow up. Findings replicate previously described hypercortisolism, partly adaptive

to chronic malnourishment and that be informative regarding long term weight restoration. Additional hormonal reactivity including DHEA-S, and testosterone has been similarly analyzed and will be described. **Significance:** Reprograming of neuroendocrine function may be in part be adaptive and may be informative regarding long term weight restoration.
Footnotes

Disclosures: T. Goltser: None. **R. Giesser:** None. **A. Shalev:** None. **R. Masarwa:** None. **Y. Martin:** None. **A. Meltzer:** None. **R. Ratzon:** None. **D. Pevzner:** None. **L. Canneti:** None. **E. Galili-Weisstub:** None. **R. Segman:** None.

Poster

P685. Hormones and Behavior: Motivation and Social Behavior

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P685.10

Topic: F.02. Behavioral Neuroendocrinology

Support: CONACYT Grant 284771

Title: Antagonism of dopamine DR2 receptors by sulpiride does not alter the metabolic profile of female and male mice

Authors: *D. VÁZQUEZ CARRILLO[1], A. OCAMPO-RUIZ[2], A. BÁEZ-MEZA[2], G. RAMÍREZ-HERNÁNDEZ[2], E. ADÁN-CASTRO[2], J. F. GARCÍA-RODRIGO[2], M. G. ORTIZ-ARBALLO[2], G. MARTÍNEZ DE LA ESCALERA[2], C. CLAPP[2], Y. MACOTELA[2];
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Abstract: Sulpiride is a second-generation antipsychotic that acts as a selective antagonist of the dopamine D2 receptor and it is commonly used in the treatment of schizophrenia, depression, and gastroparesis. In several studies, second generation antipsychotics have been linked to detrimental effects on metabolism, resulting in increased body weight and hyperglycemia in humans. It is unclear whether those deleterious effects are derived from hyperprolactinemia. In rats, there is a sex dependent effect of sulpiride on metabolic parameters. Treatment with sulpiride in female rats result in body weight gain but no alterations in glucose and insulin levels, whereas male rats do not show increased body weight but develop hyperglycemia and hyperinsulinemia. A recent study in male rats has proven that long term administration of sulpiride induces fatty liver and insulin resistance. Since there are no studies on the effects of sulpiride on the metabolic profile of mice, our aim was to evaluate the effects of sulpiride on basic metabolic parameters in both female and male mice. We used 8 weeks-old C57BL/6 mice, and treated them with sulpiride (30 mg/kg/day) during 30 days. Our results showed that sulpiride increased PRL levels to 69.8 ± 10.1 ng/mL, while in vehicle treated animals PRL levels were 9.2

± 1.2 ng/mL. Sulpiride did not increase body weight neither in females nor males. Also, fasting serum glucose, insulin and triglyceride (TG) levels were not altered in any sex. Regarding white adipose tissue, sulpiride didn't change the tissue weight, or the size and number of adipocyte cells. Taken together our results show that at this dose, regardless of sex, chronic treatment with sulpiride does not alter the metabolic profile of mice even when there is a marked hyperprolactinemic effect. A dose of 30 mg/kg/day for 30 days in male and female mice is a good model to study the effects of chronic sulpiride and hyperprolactinemia in neuroendocrine and behavioral parameters without confounding effects on metabolism.

Disclosures: D. Vázquez Carrillo: None. **A. Ocampo-Ruiz:** None. **A. Báez-Meza:** None. **G. Ramírez-Hernández:** None. **E. Adán-Castro:** None. **J.F. García-Rodrigo:** None. **M.G. Ortiz-Arballo:** None. **G. Martínez de la Escalera:** None. **C. Clapp:** None. **Y. Macotela:** None.

Poster

P701. Treatment and Drug Discovery: Mood, Anxiety, and PTSD

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P701.01

Topic: G.05. Mood Disorders

Title: Characterisation of behavioural, safety and pharmacokinetics (PK) properties of psilocybin and psilocin in rodents

Authors: *G. A. HIGGINS[1], N. CARROLL[1], M. BROWN[1], C. MACMILLAN[1], A. TOSCAN[1], J. A. ARAUJO[1,2], M. SLASSI[2], R. RAMAROHETRA[3], C. CASSIDY[3], C. TAYLOR[3], L. MAGOMEDOVA[1], I. A. M. DE LANNOY[1];
[1]InterVivo Solutions Inc, Fergus, ON, Canada; [2]MindSet Pharma Inc, Toronto, ON, Canada; [3]Transpharmation Ltd, London, United Kingdom

Abstract: Psychedelic drugs, e.g. psilocybin (PSY) are becoming widely recognized for their therapeutic potential to treat psychiatric conditions including depression and addictive disorders. PSY is rapidly metabolised to psilocin (PSI) which underlies its primary pharmacological properties. Despite PSY being evaluated in Phase 2/3 clinical trials, relatively little preclinical data for PSY exists to address issues such as PK/PD relationship between behaviours associated with CNS 5-HT_{2A}R engagement, e.g head twitch (HT; mouse) and wet dog shakes/back muscle contractions (WDS/BMC; rat). Also, the relationship between target engagement and clinically relevant plasma exposure is still poorly understood. In male C57BL/6 mice, PSY induced a dose related incidence of HT with an ED_{50} of 0.45 mg/kg SC. The incidence of HT was relatively low vs. reference 5-HT_{2A}R agonists, and confined to 10-15 min post dose. Following 1 mg/kg SC, PSY was rapidly converted to PSI, with a plasma C_{max} for PSI of 67$\pm$20 ng/ml attained at 15 min (T_{max}) and a half-life of 2.8$\pm$0.8 h; thus the duration of HT was not strictly related to drug exposure. At higher PSY doses (1-10 mg/kg SC), hypolocomotion and hypothermia emerged

(e.g. 10 mg/kg vs. vehicle: -5.6±0.5°C core body temperature; -78±3% distance travelled; both P<0.01). These effects likely impacted the expression of HT. In male Sprague-Dawley rats, PSY-induced (0.1-3 mg/kg SC) WDS/BMC were relatively mild in magnitude and not closely coupled to plasma or CSF levels of PSI. For example, following 1 mg/kg SC PSY, PSI C_{max} was 83±13 ng/ml (plasma), 35±11 ng/ml (CSF), and half-life 1.2±0.2 h (plasma), 2.5±0.8 h (CSF), yet WDS were essentially restricted to 5-20 min post treatment. The present studies suggest that while the plasma levels of PSI at ED_{50} for induction of HT (mouse) and WDS (rat) show equivalence to clinical exposures associated with psychedelic effect (~20 ng/ml; e.g. Madsen et al (2019) Neuropsychopharmacol. 44: 1328-1334), the incidence of these signs are relatively brief and not reflective of a longer half-life of PSI. Signs reflective of adverse tolerability and safety (hypolocomotion, hypothermia) emerge at higher doses and limit HT/WDS expression. These data begin to define the PD/PK relationship and safety margins for PSY in rodents and are important for benchmarking newer psychedelic drugs.

Disclosures: G.A. Higgins: F. Consulting Fees (e.g., advisory boards); MindSet Pharma. **N. Carroll:** None. **M. Brown:** None. **C. MacMillan:** None. **A. Toscan:** None. **J.A. Araujo:** None. **M. Slassi:** None. **R. Ramarohetra:** None. **C. Cassidy:** None. **C. Taylor:** None. **L. Magomedova:** None. **I.A.M. de Lannoy:** None.

Poster

P701. Treatment and Drug Discovery: Mood, Anxiety, and PTSD

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P701.02

Topic: G.05. Mood Disorders

Title: Zelquistinel (GATE-251) is an orally bioavailable novel NMDA receptor modulator that exhibits rapid and sustained antidepressant-like effects

Authors: J. S. BURGDORF[1], X.-L. ZHANG[2], P. K. STANTON[2], J. R. MOSKAL[3], J. E. DONELLO[1];
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Abstract: Nearly half of all patients with major depressive disorder (MDD) have inadequate response to antidepressants, which often take weeks to reach full effect and are associated with adverse events that can lead to discontinuation of treatment. New therapies with novel mechanisms of action and rapid onset of action would be advantageous to patients with MDD. Modulation of N-methyl-D-aspartate receptors (NMDARs) can produce rapid and sustained clinically relevant antidepression effects. Recent studies suggest that both negative and positive modulation of N-methyl-D-aspartate receptors (NMDARs) can produce rapid antidepressant effects. Here we report that zelquistinel (AGN-241751), a novel, positive NMDAR modulator,

exhibits high oral bioavailability and dose-proportional exposures in plasma and the central nervous system and produces sustained antidepressant-like effects in rodents by improving synaptic plasticity. NMDAR-mediated functional activity was measured in cultured rat brain cortical neurons (calcium imaging), hNR2A or B subtype-expressing HEK cells, and rat hippocampal and medial prefrontal cortex slices (plasticity assays). Pharmacokinetics were evaluated in rats following oral administration. Antidepressant-like effects were assessed in the rat forced swim test and the chronic social deficit mouse model. Safety/tolerability profile was assessed using PCP-induced hyperlocomotion and rotarod rodent models. Following a single oral dose, zelquistinel (0.1-100 µg/kg) produced rapid and sustained antidepressant-like effects in the rodent depression models. Antidepressant-like doses of zelquistinel also increased NMDAR function and rapidly and persistently enhanced activity-dependent synaptic plasticity (long-term potentiation), indicating zelquistinel may produce antidepressant-like effects by enhancing NMDAR function and synaptic plasticity. Positive modulation of NMDARs by zelquistinel was further exhibited in a PCP (an NMDAR antagonist)-induced hyperlocomotion assay where antidepressant-like doses of inhibited PCP-induced hyperlocomotion. Zelquistinel produces rapid and sustained anti-depressant effects by positively modulating the NMDAR and improving synaptic plasticity. Zelquistinel is currently in phase 2 development as a treatment for MDD.

Disclosures: J.S. Burgdorf: None. **X. Zhang:** None. **P.K. Stanton:** None. **J.R. Moskal:** None. **J.E. Donello:** None.

Poster

P701. Treatment and Drug Discovery: Mood, Anxiety, and PTSD

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P701.03

Topic: G.05. Mood Disorders

Title: Rapid acting antidepressants in motivation: the effect of psychedelic drugs in the rat progressive ratio task

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Abstract: Psychedelic compounds acting through the serotonin 5-hydroxytriptamine 2A (5-HT2A) receptor, have demonstrated clinical efficacy in major depressive disorder (MDD). Dysregulated motivation and reward processing have been linked to putative endophenotypes in several neuropsychiatric conditions and represent a potential transdiagnostic treatment target. Low doses of ketamine and microdoses of psilocybin have been shown to increase motivation in the rat progressive ratio task. Here, we investigated the long-term effects of ketamine and the

psychedelic 2,5-Dimethoxy-4-iodoamphetamine (DOI) on motivation.Male Sprague-Dawley rats (n=25) were trained in an operant progressive ratio task designed to measure effort-based motivation. Rats were treated with DOI (1mg/kg) and tested daily for 5 days. In a separate group of rats (n=9-10 per group), the effect of ketamine (0.1 and 0.5mg/kg) and DOI (0.2 and 1mg/kg) was investigated. Drugs were administered by subcutaneous injection. All results were compared to baseline and statistical analyses were one or two-way repeated measures ANOVAs with Fisher's LSD post-hoc.In the initial study the (1mg/kg) dose of DOI significantly decreased the number of presses ($P<0.0001$) and the breakpoint ($P<0.0001$) 30 mins after administration but showed significant increases at days 3($P<0.01$) and 5($P<0.01$) for both lever pressing and breakpoint.In the follow up study, 1mg/kg dose of DOI significantly decreased the number of presses ($P<0.0001$) and the breakpoint ($P<0.0001$) 30 mins after administration but showed significant increases at days 4($P<0.05$), 5($P<0.01$) and 6 ($P<0.05$) for lever pressing and days 5 ($P<0.01$) and 6 $P<0.05$) for breakpoint. The 0.1mg/kg dose of ketamine significantly increased the number of lever presses ($P<0.05$) and the breakpoint ($P<0.05$) 30 mins and 48 hours after administration.These data suggest biphasic temporal effects of psychedelic drugs on motivation, which may have implications for the treatment of neuropsychiatric disorders presenting with anhedonia, including MDD, or motivational dysregulation, manifesting as addiction or impulsivity. The long-lasting effects of psychedelic drugs described here, shows similarities to clinical findings demonstrating long-lasting antidepressant effects following a single dose.

Disclosures: F. Dunphy-Doherty: A. Employment/Salary (full or part-time):; Transpharmation Ireland Ltd. **D. Kozareva:** A. Employment/Salary (full or part-time):; Transpharmation Ireland Ltd. **J.R. Kelly:** None. **J. Prenderville:** A. Employment/Salary (full or part-time):; Transpharmation Ireland Ltd.

Poster

P701. Treatment and Drug Discovery: Mood, Anxiety, and PTSD

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P701.04

Topic: G.05. Mood Disorders

Support: Majda Grant
UCSD PACE Grant

Title: The aperiodic exponent: a non-invasive measure for detecting depression treatment response from resting-state EEG

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Diego, La Jolla, CA; [6]Halıcıoğlu Data Sci. Inst., La Jolla, CA; [7]Kavli Inst. for Brain and Mind, La Jolla, CA

Abstract: Treatments for Major Depressive Disorder (MDD) include medication, therapy, and stimulation procedures like electroconvulsive therapy (ECT). These interventions target a variety of neurophysiological processes including neurotransmitter imbalances or pathological electrophysiological activity. Ultimately, these therapies aim to restore physiological activity and ameliorate depressive symptoms. However, research investigating the precise mechanisms behind these treatments remains inconclusive, often due to the lack of reliable clinical indicators. For instance, frontal alpha asymmetry, the most common electrophysiological indicator of depressive disorders in clinical contexts, has been repeatedly demonstrated to be invalid (van der Vinne et al, 2017; Kolodzeij et al, 2021).

Here, we evaluated electrophysiological signatures of the effects of ECT on neural activity in a unique dataset of 10 clinical MDD patients receiving ECT treatment. In this study, resting-state EEG data and clinical symptom ratings (QIDS) were collected from MDD patients over a 12-session ECT treatment. Data were collected before and after the first, fourth, eighth, and twelfth ECT session to permit analysis of short term effects within a single session, and long-term effects over the course of treatment. We characterize these data in the frequency domain using spectral parameterization, a cutting-edge tool with the capacity to measure features of both periodic and aperiodic neural activity (Donoghue et al, 2020).

Our results reveal that aperiodic activity significantly changes with ECT treatment. Specifically, the aperiodic exponent increases in frontal electrodes acutely following an ECT session ($p < 0.00001$). Importantly, this change in the aperiodic component of the power spectrum results in an apparent reduction in canonical alpha (8-13 Hz) band power. However, alpha oscillatory power above the aperiodic component does not significantly change ($p = 0.694$), indicating no change in genuine oscillatory activity. Additionally, on a longer timescale, the aperiodic exponent also increases (becomes less flat) progressively over the course of treatment ($p = 0.031$). We further generalize these findings by comparing aperiodic activity changes in a broader MDD population undergoing a sertraline treatment by leveraging the EMBARC dataset. These results identify aperiodic activity as a potential clinical indicator for depression treatment and suggest that the insufficiency of indicators like frontal alpha asymmetry is potentially a result of the reliance on traditional methods of spectral analysis.

Disclosures: S. Smith: None. **D. Printz:** None. **B. Voytek:** None. **M. Soltani:** None.

Poster

P701. Treatment and Drug Discovery: Mood, Anxiety, and PTSD

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P701.05

Topic: G.05. Mood Disorders

Support: Caltech Rothenberg Innovation Initiative
Della Martin Foundation
MH12083
GM123582
HHMI

Title: A Family of Genetically Encoded Biosensors for Rapidly Acting Antidepressants (RAADs) inside Neurons

Authors: K. BERA[1], E. LIN[1], E. J. FINE[1], Z. BLUMENFELD[1], A. V. SHIVANGE[1], B. N. COHEN[1], J. S. MARVIN[2], C. H. KIM[1], J. H. WANG[1], P. DESHPANDE[1], L. L. LOOGER[2], H. A. LESTER[1];
[1]Div. of Biol. and Biol. Engin., Caltech, Pasadena, CA; [2]Janelia Res. Campus, Howard Hughes Med. Inst., Ashburn, VA

Abstract: Rapidly acting antidepressants (RAADs) partially relieve depression in < 1 day and have sustained antidepressant effects for several days. This onset is 10- to 100-fold faster than selective serotonin and norepinephrine reuptake inhibitors (SSRIs and SNRIs). RAADs have various structures. Some RAADs are ketamine enantiomers (R-Ket/S-Ket), metabolites [(2R, 6R)-HNK/ (2S, 6S)-HNK], or analogs such as methoxetamine (MXE), and radafaxine (Rad). Other RAADS such as scopolamine (Scop), are best known as muscarinic antagonists and are structurally unrelated to ketamine. The mechanism(s) of RAAD action are largely unknown at levels ranging from their molecular target, their signaling pathway, the cell types they affect, and other circuits they may influence. Thus, it is important to visualize and quantify RAADs inside subcellular regions of neurons.
Therefore, we developed the "intensity-based RAAD-Sensing Fluorescent Reporter" [iRAADSnFR] genetically encoded biosensor family for RAADs. This biosensor family structurally resembles a previously published nicotine biosensor: a fusion between a mutated bacterial periplasmic binding protein OpuBC, interdomain linkers, and circularly permuted green fluorescent protein (GFP). Biosensor mutations are created by site-saturated mutagenesis and are tested with, high-throughput fluorescence screens, and fluorescence measurements with their purified protein form, and computational docking models. We have mutated residues at the binding site to produce selectivity and pharmacologically relevant sensitivity to various RAADs.
iRAADSnFR - EC_{50} - $\Delta F_{max}/F_0$: iS-KetSnFR - 164 nM - 3.2; iS-HNKSnFR - 110 nM - 3.6; iR-KetSnFR - 130 nM - 4.0; iR-HNKSnFR - 1.1 µM - 3.3; iMXESnFR - 47 nM - 8.6; iRadSnFR - 1.6 µM - 6.3; iScopSnFR - 510 nM - 2.8.
We incorporate additional sequences to the iRAADSnFR constructs to target the sensors to the plasma membrane (PM), cytoplasm, peroxisome, mitochondria, endoplasmic reticulum (ER), nucleus, Golgi, lysosome, and synaptic vesicles. Fluorescence dose-response experiments to test the efficacy of various iRAADSnFRs in live mammalian cells have progressed. Fluorescence microscopy shows that, at the nM concentrations relevant to the antidepressant action, RAADs enter and leave the various organelles within a few seconds, attaining extracellular levels. We also recently utilized adeno-associated virus (AAV) capsids to facilitate efficient and noninvasive iRAADSnFR gene transfer to the central and peripheral nervous systems which may help us unravel RAADs' mechanism of action in zebrafish and mice.

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Poster

P701. Treatment and Drug Discovery: Mood, Anxiety, and PTSD

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P701.06

Topic: G.05. Mood Disorders

Support: R01MH123093-01S1
T32GM007518

Title: Immune Phenotypes of Oligodendroglial-Lineage Cells in MDD and in Response to Microglial-Derived Inflammation

Authors: *M. M. MADEIRA, A. G. KOKKOSIS, S. H. KLEIN, Z. M. HAGE, S. E. TSIRKA; Pharmacol. Sci., State Univ. of New York, Stony Brook, Stony Brook, NY

Abstract: Background Major Depressive Disorder (MDD) is a complex psychiatric disorder affecting more than 300 million people worldwide. Though the mechanisms underlying MDD are not fully understood, a subset of depressive patients can manifest chronic neuroinflammatory responses. Brain-imaging studies in MDD patients have provided evidence of white matter reductions and such changes are hypothesized to lead to disruption of Oligodendroglial-Lineage cells (OLN) homeostatic mechanisms, resulting in destabilization of emotional/cognitive circuitry. The present goal of the study is to investigate whether these histopathological alterations are linked with chronic inflammation through single-nucleus transcriptomics of MDD patients and using *in vitro* techniques.**Methods** *Bioinformatics:* Raw counts were downloaded from GEO (GSE144136), and expression objects created using Seurat. OLN were subsetted, clustered (using UMAP), and annotated based on their expression of OLN markers. Pseudotime trajectory analysis was conducted using Moncle3. *In vitro:* OPCs and Microglia werederived from Neonatal (P1-P3) mice. To induce an M1-like polarization in Microglia, the cells were treated with LPS. Conditioned Media was then collected and used to treat OPCs at multiple time points and concentrations. To assess dysfunction in proliferation and the adoption of immune phenotypes, immunofluorescent studies were conducted. **Results** Separate clustering of MDD and Control samples yielded four similar clusters labeled based on their geneexpression as: OPCs, Committed OPCs, Immature Oligodendrocytes (OLs), and Mature OLs. One MDD-specific cluster was also obtained and labeled as Immune OLs (ImOL), given its expression of immune genes, such as: *CD74*, and *P2RY12*. Further analysis of the ImOL cluster revealed expressionof markers associated with later phases of OLN progeny. Finally, a predictive model of pseudotime trajectoryrevealed alterations in the progression of OLN progeny in MDD

samples. We then sought to identify ImOL in culture. MHCII and P2RY12 were examined in conjunction with OLN markers (PDGFRα and MBP). To assess whether cytokines derived from activated microglia affected the proliferation of OPCs, Ki67 was used. Interestingly, the presence of inflammatory cytokines from microglia caused significant changes in the OPCs, rendering the ImOL as potential contributors to pathophysiology.**Conclusions** Together, our computational and *in vitro* work shows that OLN can swiftly respond to inflammatory processes arising from innate immune cells, and adopt an immune-like phenotype, potentially contributing to the white matter disturbances observed in MDD.

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Poster

P701. Treatment and Drug Discovery: Mood, Anxiety, and PTSD

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P701.07

Topic: G.05. Mood Disorders

Title: Dietary aryl hydrocarbon receptor ligands as potential antidepressants

Authors: *C. MADISON[1], G. ALBRECHT[1], J. KUEMPEL[1], L. HILLBRICK[1], S. SAFE[2], A. JAYARAMAN[1], R. CHAPKIN[3], S. EITAN[4];
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Abstract: Depression is a major public health issue that has significantly worsened since the beginning of the COVID-19 pandemic. Current pharmaceutical treatments for depression, such as selective serotonin reuptake inhibitors (SSRIs), are sometimes ineffective and may have unwanted side effects that interfere with patient compliance. Tryptophan (Trp) is an amino acid precursor for serotonin, and altered Trp metabolism is believed to contribute to the psychopathology of depression. Decreased Trp levels have been found in patients who have depression and those who have died by suicide. Gut microbiota play a role in Trp metabolism, and gut flora composition has been linked to depressive disorders in humans and depressive-like behavior in rodents. The Aryl-hydrocarbon receptor (AhR) is an intracellular transcription factor present in various somatic and immune cells, and it is known to be activated by certain Trp metabolites. It has been hypothesized that AhR may play a role in the gut-brain-immune axis in multiple disease states including neurological disorders. Anhedonia is a hallmark sign of Major Depressive Disorder (MDD) and is one of the two criteria necessary for diagnosis under the Diagnostic and Statistical Manual of Mental Disorders (5th ed; DSM-5). The current study induced an anhedonia-like state in female C57BL/6 mice using unpredictable chronic mild stress

(UCMS). Diindolylmethane (DIM) and 1,4-dihydroxy-2-naphthoic acid (DHNA) are plant- and microbe-derived AhR ligands, respectively. For three weeks prior to UCMS mice were fed one of two ligands for the AhR, DIM or DHNA; a second group was subjected to two weeks UCMS before ligand administration began. Both compound successfully prevented and reversed anhedonia-like behavior as measured by the sucrose preference test (SPT). Furthermore, an isomer of DHNA which is inactive with respect to AhR, did not prevent the UCMS-induced anhedonia-like behavior. Additionally, both DIM and DHNA did not significantly alter anxiety levels and did not cause spatial learning deficits. The results suggest dietary AhR ligands as potential antidepressants. Future studies are necessary to elucidate the role of AhR in depression-like states and the underlying mechanisms.

Disclosures: C. Madison: None. **G. Albrecht:** None. **L. Hillbrick:** None. **S. Safe:** A. Employment/Salary (full or part-time):; Texas A&M University. **A. Jayaraman:** None. **R. Chapkin:** A. Employment/Salary (full or part-time):; Texas A&M University. **S. Eitan:** None.

Poster

P701. Treatment and Drug Discovery: Mood, Anxiety, and PTSD

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P701.08

Topic: G.05. Mood Disorders

Support: NIMH grant 5K23MH100640-04

Title: Defining Neural Signatures of Auditory Information Processing in Youth with Obsessive Compulsive Disorder

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Abstract: Obsessive compulsive disorder (OCD) is one of the most common psychiatric disorders and involves obsessive thoughts or compulsive behaviors which may be related to abnormalities in information processing involving an impaired inhibitory process. While the average age of onset is in late adolescence, OCD is also commonly diagnosed in children. Resting state gamma activity abnormalities have been observed in adults with OCD as well as other psychiatric disorders. Reduced local circuit inhibition leading to inability to synchronize neural responses to sensory input is hypothesized to lead to increased non-specific gamma activity. Further characterization of pediatric pathophysiology is necessary to understand patterns of disease progression and allow for development of neurophysiological markers that can assist in diagnosis.
Magnetoencephalography (MEG) is suitable for examining high frequency, low amplitude

activity such as gamma band oscillations. First, we examined whole brain resting state gamma activity in 10 OCD and 10 healthy youth who ranged in age from 11-17. We hypothesized based on the adult literature that absolute gamma power would be elevated in pediatric subjects with OCD compared to healthy controls. While we found no differences in mean and absolute gamma power, minimum absolute gamma power was significantly higher in OCD compared to healthy subjects.
As described above, symptoms of OCD can be related to abnormalities in sensory processing. Our previous auditory evokes response study demonstrated a delayed latency of auditory evoked responses in pediatric subjects with OCD. Given that gamma is the primary working frequency of the auditory system, it is possible to drive the sensory cortex with stimuli oscillating at gamma frequencies and examine its ability to synchronize cortical activity. A subset of the 5 healthy subjects and 5 with OCD in whom resting state activity was examined were exposed to an auditory chirp stimulus generated using a 1000Hz tone that was amplitude modulated by a sinusoid linearly increasing in frequency from 0-100Hz over 2 seconds. This stimulus has been used to demonstrate a reduced ability of subjects with other developmental disorders (Fragile X Syndrome and Autism Spectrum Disorder) to synchronize neural activity at higher frequencies in the gamma range. We hypothesize that there may be a common developmental phenotype amongst these disorders and that subjects with OCD will exhibit similar deficits in neural synchronization in the gamma frequency range when exposed to the auditory chirp stimulus. Characterizing this response could lead to development of a diagnostic tool for early detection of OCD.

Disclosures: D. Cass: None. **J. Meyer:** None. **M. Korostenskaja:** None. **E. Harris:** None. **I. Basu:** None.

Poster

P701. Treatment and Drug Discovery: Mood, Anxiety, and PTSD

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P701.09

Topic: G.05. Mood Disorders

Title: The selective GPR139 agonist TAK-041 reverses anhedonia and social interaction deficits in rodent models related to negative symptoms in Schizophrenia

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Abstract: Negative symptoms in schizophrenia (SCZ) are poorly treated and interfere with the function of patients. Studies focusing on structural and functional imaging and non-invasive

electrophysiology implicate perturbations of the frontocortico-temporal circuits and disruption of cortico-striatal loops to negative symptoms in SCZ. GPR139 is an orphan GPCR that is specifically expressed in the CNS and enriched in the habenula, a brain structure involved in reward and motivation. Structural and functional alterations of the habenula have been found in SCZ patients, who show deficits in feedback processing and lack habenula activation in response to negative outcomes. Rodent experiments demonstrate the involvement of direct projection from the cortex to the habenula controlling social behavior. Alterations in habenula activity have been correlated with depression, and normalization of aberrant habenula activity has been proposed as therapeutic strategy to reverse anhedonia. Building on data previously reported, we further explored modulation of GPR139 receptors and habenula circuitry *in vivo* as a novel mechanism to treat negative symptoms in SCZ. The selective GPR139 agonist, TAK-041, increased cFOS expression in the habenula in wild type mice, but not in GPR139 knock out mice. No desensitization of cFOS in the habenula was observed after chronic dosing of TAK-041. TAK-041 reduced amphetamine- and nicotine-induced dopamine release in the NAc in rats. In the rat unpredictable chronic mild stress (uCMS) model, acute and chronic treatment with TAK-041 reversed the anhedonic and anxiolytic effects, as measured by the forced swim test and the novelty-suppressed feeding test, respectively. Additionally, uCMS-exposed rats developed a disrupted circadian regulation of corticosterone secretion, which was also normalized by TAK-041 treatment. Furthermore, TAK-041 reversed the uCMS-induced atrophy in the basal dendrites of pyramidal neurons in the hippocampus and the uCMS-induced hypertrophy of medium-spiny neurons in the NAc. These results suggest a potential benefit of TAK-041 in the treatment of anhedonia and possibly depression. TAK-041 also reversed social interaction (SI) deficits in the maternal immune activation poly-I:C model of SCZ, the subchronic PCP-SI model and in Balb/C and BTBR mice. The GPR139 agonist TAK-041 is proposed as a modulator of habenula circuitry to treat negative symptoms in SCZ based on efficacy in reversing anhedonia and social interaction deficits in multiple rodent models related to negative symptoms in SCZ.

Disclosures: H. Schiffer: A. Employment/Salary (full or part-time):; Takeda Pharmaceuticals. **J. Atienza:** A. Employment/Salary (full or part-time):; Takeda Pharmaceuticals. **H. Reichard:** A. Employment/Salary (full or part-time):; Takeda Pharmaceuticals. **V. Mulligan:** None. **J. Cilia:** None. **H. Monenschein:** A. Employment/Salary (full or part-time):; Takeda Pharmaceuticals. **D. Collia:** None. **W.J. Ray:** None. **G. Kilpatrick:** None. **N. Brice:** None. **M. Carlton:** None. **S. Hitchcock:** A. Employment/Salary (full or part-time):; Takeda Pharmaceuticals. **G. Corbett:** None. **R. Hodgson:** A. Employment/Salary (full or part-time):; Takeda Pharmaceuticals.

Poster

P701. Treatment and Drug Discovery: Mood, Anxiety, and PTSD

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P701.10

Topic: G.05. Mood Disorders

Support: RFMH: 903- 2940A

Title: Tianeptine, but not fluoxetine, decreases avoidant behavior in a mouse model of early developmental fluoxetine exposure

Authors: *E. A. PEKARSKAYA[1], E. S. HOLT[2], J. A. GINGRICH[1,3], M. S. ANSORGE[3,4], J. A. JAVITCH[2,4,5], S. E. CANETTA[2,3];
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Abstract: Depression and anxiety are two of the most common mental health disorders, often co-occurring and with overlapping symptoms and treatments. Unfortunately, roughly half of patients do not respond to frontline selective serotonin reuptake inhibitors (SSRIs), or other monoamine targeting treatments. Currently, finding the most effective treatment for an individual is a process of trial and error. To better understand how disease etiology may predict treatment response, we studied mice exposed developmentally to the SSRI fluoxetine (FLX). Human epidemiological data indicates that patients with developmental exposure to FLX have a higher risk of both adult affective disorders and treatment resistance to SSRIs. Similarly, developmentally exposed mice show the murine equivalent of anxiety- and depression-like symptoms in adulthood and are resistant to subsequent FLX treatment. Therefore, we investigated whether tianeptine (TIA), an atypical antidepressant which exerts its therapeutic effects through the mu-opioid receptor instead of directly targeting monoaminergic systems, would be more effective in this model.C57BL/6J (C57) male and female pups were injected with either FLX (10 mg/kg, i.p) (n = 39, 18m, 21f) or vehicle (n = 31, 14m, 17f) from postnatal (PN) day 2 to 11, a period in which mouse brain development parallels that of the third trimester of a human pregnancy. Prior work established that adult 129SvEv (129) mice exposed to FLX in this time period showed increased avoidant behaviors, which are common in mouse models of anxiety and depression. We performed baseline testing in adulthood and confirmed that C57 PN-FLX animals share a similar avoidant phenotype to that reported in 129 PN-FLX mice. As sex did not significantly impact the variables measured, we report the data from the sexes combined. Animals were then pseudorandomly assigned into adult administration groups, and received either FLX (18 mg/kg) (PN-FLX = 20, PN-VEH = 16) or vehicle (PN-FLX = 19, PN-VEH = 15) in their drinking water for 3 weeks. We evaluated the effect of adult FLX treatment on two tasks that measure avoidant behavior - the open field and novelty suppressed feeding (NSF) tasks. This treatment failed to improve, and even exacerbated, avoidance in PN-FLX mice. By contrast, when the same animals later underwent chronic treatment with TIA (30 mg/kg, 2x/day, i.p.), avoidance behavior decreased in both the open field and NSF tasks. Overall, this demonstrates that TIA may be a promising alternative treatment to typical antidepressants, especially in patients whose serotonergic system has been altered by in utero exposure to SSRIs.

Disclosures: E.A. Pekarskaya: None. **E.S. Holt:** None. **J.A. Gingrich:** None. **M.S. Ansorge:** None. **J.A. Javitch:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dr. Javitch is co-inventor on patents held by Columbia University on tianeptine analogs.. **S.E. Canetta:** None.

Poster

P701. Treatment and Drug Discovery: Mood, Anxiety, and PTSD

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P701.11

Topic: G.05. Mood Disorders

Support: Vulnerable Brain Project
Dean's Undergraduate Research Fund of NYU

Title: Individual differences in plasticity evoked by ketamine in a mouse-model of Anorexia Nervosa: Analysis of drebrin, an F-actin binding protein in postsynaptic spines of the medial prefrontal cortex

Authors: *R. TEMIZER[1], C. J. AOKI[2], Y.-W. CHEN[3];
[1]New York Univ. Ctr. For Neural Sci., New York, NY; [2]Ctr. Neural Sci., [3]Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Anorexia Nervosa (AN) is a mental illness with highest rates of mortality and relapse, and no approved pharmacological treatment. Chen, Aoki et al. (2018) used the Activity-Based Anorexia (ABA) mouse model of AN to examine the ameliorative role of ketamine (KET) for AN, as KET is used to treat anxiety and depression, both of which are comorbidities of AN. It was shown that 30 mg of KET is effective in enhancing food consumption and reducing the maladaptive behavior of excessive running, when administered during mid-adolescence (P42) with noticeable individual differences in the drug's efficacy. Like its action as an antidepressant, KET's ameliorative effect upon ABA was long-lasting (>2 weeks), potentially protecting against relapse and possibly promoting structural plasticity, such as spinogenesis and synaptogenesis. We hypothesized that KET's ameliorative effects upon ABA involves altered expression of drebrin, an F-actin-binding protein that up-regulates in expression during developmental phases of robust spinogenesis, such as mid-adolescence. Moreover, since drebrin was shown previously to mediate the activity-dependent trafficking of NMDARs into and out of dendritic spines and correlate with ABA vulnerability through enhanced anchoring of NMDARs post-synaptically, we surmised that KET could contribute to ABA resilience through reduced localization of drebrin within postsynaptic spines. To test this hypothesis, we conducted electron microscopic immunocytochemistry (EM-ICC) to quantify drebrin immunoreactivity (ir) within postsynaptic spines of mice that underwent two cycles of ABA induction and received 30 mg (efficacious) versus 3 mg (non-efficacious) of KET during the first ABA induction in mid-adolescence. The Prefrontal Cortex (PFC) is the most important brain region for decision-making and layer 1 of PFC has been implicated as a site of action of KET. We therefore focused our analysis upon this brain region.EM-ICC revealed that the level of drebrin at plasma membrane correlated negatively with the percent of body weight restored of mice 6 days after the end of the food

restricted (FR) period (equal to 8 days after having received 30 mg of KET during FR) (r=-0.9, p=0.078, N=4). This suggests that those animals with the highest levels of plasma-membranous drebrin were the individuals least able to restore their body weights (least resilient). In contrast, synapse density did not correlate with the extent of body weight restoration (r=-.07, p=0.9, N=4), indicating no detectable effect of KET on spinogenesis. We will continue with these analyses by increasing sample size, and comparing to drebrin-ir of animals that received 3 mg of KET.

Disclosures: R. Temizer: None. **C.J. Aoki:** None. **Y. Chen:** None.

Poster

P701. Treatment and Drug Discovery: Mood, Anxiety, and PTSD

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P701.12

Topic: G.05. Mood Disorders

Support: U.S. Highbush Blueberry Council

Title: Effects of whole blueberry supplementation on symptoms of depression and anxiety in a rural Louisiana population: behavioral outcomes and inflammatory cytokines from a double-blind, placebo-controlled, crossover study

Authors: *K. VENABLE[1], D. KELLEY[1], H. J. CRULL[1], P. EBENEZER[1], S. O'BRYAN[1], A. JEANSONNE[2,3], C. C. LEE[1], S. JONES[4], V. VATSAVAYI[5], J. FRANCIS[6];
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Abstract: Blueberries are densely nutritional with high antioxidant capacity and anti-inflammatory properties demonstrating beneficial effects on health and multiple disease states in numerous preclinical and clinical studies. Depression and anxiety are prevalent and costly disorders, with increased incidence observed during the pandemic. Emerging evidence supports a relationship between depression and the immune system, with chronic, low-grade, systemic inflammation observed in a subset of individuals diagnosed with depression; connections between anxiety and the immune system are less clear at this time. To understand how blueberries might support physiological health and emotional wellbeing of individuals diagnosed with depression or anxiety, we collaborated with a Louisiana rural health clinic system, Louisiana Health Care Practitioners (LHCP), and designed and implemented a randomized, placebo-controlled, crossover study at 2 LHCP sites. This study consisted of two 12-week arms with baseline, mid, and post treatment assessments in each arm and a 4 week washout period between; in arm 1, participants were randomly assigned to either whole, freeze-dried blueberry

powder or a matched placebo control powder which they suspended in water and ingested (24 g) each day (for 12 weeks). At crossover, those who were assigned to blueberry in arm 1 switched to placebo in arm 2 and vice versa. At all time points we collected blood samples and MDI and GAD7, self-report measures of depression and anxiety symptoms, respectively. At baseline and post assessments, we also administered the SIGH-D, a verbal interview utilizing the Ham-D scale and the IDSC. To investigate cytokine expression in participant plasma samples, we used Luminex multiplex assays targeting IL-1β, TNF-α, Il-6, IL-10, IFNγ, and CRP. We confirmed no measured behavioral correlation to CRP, but found a weak correlation to IL-1β; certain individuals' depressive and anxious symptoms were more tightly correlated inflammatory cytokines. Some individuals' depression symptoms were ameliorated in the blueberry condition ("responders"), relative to placebo, whereas others showed no response. Blueberry treatment attenuated anxiety symptoms compared to placebo, but this effect was only observed in the first arm. We plan to use mixed effects modeling to analyze the relationship between blueberry treatment, inflammatory cytokine expression, and behavioral outcomes. This pilot study appears to support the growing momentum toward more personalized forms of nutrition and medicine; this need is especially prominent in psychiatric realms and for disorders as complex as depression and anxiety.

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Poster

P740. Alcohol: Circuitry and Neurophysiology of Addiction III

Location: Virtual Only

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Program #/Poster #: P740.01

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant AA023786
NIH Grant AA007462
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NIH Grant AA022821

Title: Dorsal medial prefrontal cortex neural firing correlates of compulsive drinking in alcohol preferring P rats

Authors: N. M. TIMME[1], B. MA[1], D. LINSENBARDT[2], E. CORNWELL[1], T. GALBARI[1], C. LAPISH[1];

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Abstract: Drinking alcohol despite negative consequences (so called "compulsive drinking") is a hallmark of an advanced alcohol use disorder (AUD). At this stage, treatments tend to be less effective, so understanding the cause of this behavior is critical to identify more effective treatment approaches. To better understand this phenomenon, we recorded neural activity in dorsal medial prefrontal cortex (dmPFC, a brain region known to be involved in decision-making) during challenged drinking in adult male Wistar rats (control) and P rats (bred to prefer alcohol, model for genetic risk for excessive drinking). These animals were acclimated to alcohol in home cage and then trained to consume alcohol in a 2-way cued access protocol (2CAP). Behavioral and neural firing data were gathered during the 2CAP task in maintenance and quinine adulterated alcohol (0.1 g/L, challenged drinking test) sessions. P rats continued to consume quinine adulterated alcohol (compulsive drinking) while Wistar rats showed a dramatic decrease in consumption (non-compulsive drinking). Analysis of neural firing in P rats demonstrated that dmPFC representations of seeking were strengthened, while representations of behavioral control variables (e.g., approaching the sipper and drinking) were weakened. Conversely, Wistars showed a strengthening of behavioral control variables during challenged drinking. Furthermore, this Wistar neural response appeared to be driven by increased neural excitation. Therefore, we increased excitation in dmPFC in P rats using a chemogenetic technique, which resulted in a suppression of the progression of compulsive drinking. These studies further elucidate dmPFC's role in compulsive drinking.

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Poster

P740. Alcohol: Circuitry and Neurophysiology of Addiction III

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Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P740.02

Topic: G.09. Drugs of Abuse and Addiction

Title: Neural correlates of transdiagnostic psychiatric symptom factors in Veterans with alcohol use disorder

Authors: *L.-T. TENEKEDJIEVA[1], A. N. GOLDSTEIN-PIEKARSKI[1,3], K. H. MACNIVEN[2], M. MADORE[1,3], M. KAUR[1], L. WILLIAMS[1,3], C. PADULA[1,3];
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Abstract: Over the past decades, researchers have made great strides in delineating the neurobiological correlates underlying alcohol use disorder (AUD). However, individuals with AUD have high rates of psychiatric comorbidities, which are critical to consider to fully understand the heterogeneity of AUD. The specific symptoms underlying depression, anxiety, and PTSD overlap across diagnoses, highlighting the need to obtain a transdiagnostic understanding. Thus, the current study aims to identify psychiatric symptom factors across diagnostic categories using a data-driven approach and to determine how they relate to the neural circuitry of addiction. A total of 80 Veterans with AUD (22W) completed a psychodiagnostic interview, self-report questionnaires and a 2-hour MRI session. The MRI session included functional MRI scans while participants completed four tasks: emotion processing of faces, monetary incentive delay, alcohol cue reactivity, and a go-no go task. To understand transdiagnostic symptom compositions, we performed factor analysis on self-reported symptoms measured by the Depression, Anxiety and Stress Scale (DASS) and the PTSD Checklist scale (PCL-5). Symptom component scores were calculated based on the factor solution and neural correlates with a-priori defined regions of interest in the four tasks were assessed. Factor analysis revealed four symptom components corresponding to trauma distress, negative emotionality, somatic anxiety and hyper-arousal. Significant associations were revealed between trauma distress score and reduced reward regions activity (L. postInsula) and increased cognitive control activity (dACC). Negative emotionality was related to lower activation in reward regions (R. putamen, L. postInsula and R. dlPFC) and alcohol cue reactivity regions (R. dlPFC, L. mOFC), but higher activation in cognitive control (R. dlPFC) and threat regions (antInsula). Somatic anxiety was associated with decreased activation in reward (R. putamen, R. antInsula) and cue reactivity (R. dlPFC) and increased activation in threat networks (R. antInsula). Higher hyper-arousal scores related to lower activation in reward regions (R. putamen, R. antInsula, L. postInsula, R. antIPL) and increased activation in threat networks (R. antInsula). These findings offer transdiagnostic symptom factors that are differentially associated with neural function and guide us towards a brain-based classification of psychiatric dysfunction in AUD. Future studies will assess the relationship between symptom factors and relapse rates, with the goal of understanding the interplay of relapse risk and mood and trauma symptoms.

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Poster

P740. Alcohol: Circuitry and Neurophysiology of Addiction III

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Program #/Poster #: P740.03

Topic: G.09. Drugs of Abuse and Addiction

Support: AA024109 (FWH)

Title: Inhibiting beta-noradrenergic receptors suppresses compulsion-like alcohol drinking more effectively than alcohol-only intake, and not through the Anterior Insula

Authors: T. DE OLIVEIRA SERGIO, S. WEAN, S. KATNER, ***F. HOPF**;
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Abstract: Compulsion-like alcohol drinking (CLAD), where consumption continues despite negative consequences, is a major obstacle to treating alcohol use disorder. Norepinephrine receptor (NER) signaling in forebrain cortical regions have been implicated in adaptive responding under stress, which is conceptually similar to compulsion-like responding (adaptive responding despite the presence of stress or conflict). We have previously shown that anterior insula (aINS)-to-brainstem connections inhibited CLAD but not alcohol-only drinking (AOD), while alpha-1 NERs (inhibited systemically or within aINS with prazosin) regulate compulsion-like intake and AOD (de Oliveira Sergio et al., 2021 *Neuropsychopharm*). Here, we examine the importance of beta NERs for CLAD and AOD. While alpha1-NER inhibition suppresses CLAD and AOD equivalently at effective doses of prazosin, the beta-NER blocker propranolol shows a different plan, where a middle dose (5 mg/kg) inhibits CLAD but not AOD, while a higher dose (10 mg/kg) inhibits both CLAD and AOD and a lower dose (2.5 mg/kg) has no effects. In addition, combining a subthreshold, ineffective dose of propranolol (2.5 mg/kg) with a subthreshold, ineffective dose of prazosin (0.25 mg/kg) significantly reduces both CLAD and AOD, supporting the use of a combination therapy in human problem alcohol drinkers to decrease alcohol drinking while reducing other side effects. To investigate brain regions where propranolol acts to suppress alcohol drinking, we first examined propranolol effects in the aINS. Surprisingly, and unlike alpha1-NERs, inhibiting beta-NERs in the aINS over a broad dose range (1-10 ug/side) had no effect on CLAD or AOD. We are presently testing propranolol in the mPFC, since some evidence (Funk and Stewart, 1996) suggests that some autonomic functions may be regulated by beta-NERS in mPFC more than aINS. We are also examining inhibitors specific to particular beta-NERs. Together, our findings suggest (1) that different aspects of the noradrenergic system can differentially regulate compulsion-like vs alcohol-only drinking, with beta-NERs perhaps more selective for CLAD than alpha1-NERs, (2) that CLAD is likely regulated by different receptors across different regions of the salience network, and (3) that alpha1-NERs, beta-NERs, and a combination therapy using subthreshold doses, all may have clinical utility in treating problem alcohol drinking in humans. Supported by AA024109 (FWH).

Disclosures: T. De Oliveira Sergio: None. **S. Wean:** None. **S. Katner:** None. **F. Hopf:** None.

Poster

P740. Alcohol: Circuitry and Neurophysiology of Addiction III

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Program #/Poster #: P740.04

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH/NIAAA AA013983
NIH/NIDA DA031734

Title: To fight or not to fight: activation of neural circuits in VTA-mPFC for aggressive motivation and decision making after ethanol consumption in a novel murine model

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Abstract: Assessing the neural mechanisms for the motivation to fight remains a challenge for preclinical models, particularly with inbred strains of mice. The effects of alcohol on the decision to engage in aggressive behavior in preference over sociosexual contact is hypothesized to be based on medial Prefrontal Cortex-Ventral Tegmental Area (PFC-VTA) connections that differ from those necessary for the actual execution of these pro- and anti-social behaviors. In a newly developed procedure we studied social choice in male C57BL/6J mice based on preferentially seeking access to sociosexual interactions with a female partner vs. attacking an intruder male. While deciding to engage in aggressive vs. sociosexual behavior, neural activation was studied with c-fos immunohistochemistry in cortical, amygdaloid and tegmental regions of interest. Adult male B6 mice demonstrated sexual competence by siring offspring. During repeated confrontations with an intruder male in their home cage they exhibited pursuit, threat and attack behavior within less than 5 min. The mice were then conditioned to respond at an illuminated operandum in an experimental chamber attached to their home cage; completion of 10 responses (Fixed Ratio 10; FR10) was reinforced by access to either a female or a male intruder which were presented in the resident's home cage. Brains were harvested at the moment of choice between the concurrently available aggressive and sociosexual options. Immuno-stained c-fos in 10 brain regions of interest was analyzed by fluorescence microscopy. The effects of ethanol self-administration were examined on choice for aggressive behavior and on c-fos expression in the mPFC-VTA pathway. Six out of 50 mice chose to complete consistently the FR 10 response option that was reinforced by aggressive behavior toward a male intruder, while not responding on the concurrently available option that was reinforced by sociosexual contact with a female. Self-administered alcohol (1-1.5 g/kg) increased responding for the aggressive option in most mice. When choosing the aggressive, but not the sociosexual option, the prelimbic area of the PFC revealed more c-fos activity prompting more detailed inquiry.

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Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA F31 AA028410

Title: Alcohol modulates basolateral amygdala network activity implicated in anxiety

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Abstract: Alcohol is one of the most widely used drugs in the United States with approximately 85% of adults reporting drinking in their lifetime. Although there are many reasons people drink, one strong motivating factor is anxiety. Indeed, alcohol use disorder and mood disorders, like generalized anxiety disorder, are highly comorbid. The intimate relationship between alcohol and anxiety is partly controlled by the basolateral amygdala (BLA), which plays a role in expressing anxiety-like behaviors, as well as mediating the negative aspects of alcohol withdrawal. However, it is unclear how alcohol changes activity in the BLA to influence anxiety-like behavior. We hypothesized that alcohol could influence network oscillations in the BLA which in turn influences behavior. Network oscillations, created by rhythmic and synchronous neuronal activity, can exist at different oscillatory frequencies that are associated with different behavioral states, like fear and anxiety. Therefore, we characterized the effects of alcohol on network oscillations by recording local field potentials (LFPs) in male and female C57BL/6J mice after acute and repeated exposure to alcohol (1 g/Kg I.P.). We demonstrate that alcohol is capable of altering BLA network states in a sex dependent manner. Further, we have shown that GABAergic parvalbumin (PV) interneurons in the BLA are critical for generating these oscillations and can shift between specific frequencies to influence freezing behavior in mice. Our lab has shown that δ-subunit containing $GABA_A$ receptors are specifically expressed on PV interneurons in the BLA and others have demonstrated that these receptors are uniquely sensitive to low levels of alcohol. Therefore, we hypothesized that alcohol may modulate BLA oscillations via δ-$GABA_A$ receptors on PV interneurons. To test this hypothesis, we repeated the experiment cited above in male and female global delta knockout (KO) mice. We found subtle differences in the effects of alcohol in the KO mice, suggesting delta is not solely responsible for alcohol's effects on BLA oscillations.

Disclosures: A. DiLeo: None. **S. Ha:** None. **P. Antonoudiou:** None. **J.L. Maguire:** None.

Poster

P740. Alcohol: Circuitry and Neurophysiology of Addiction III

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P740.06

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01 AA028228

Title: Kappa-opioid receptor function in the nucleus accumbens shell differentially affects ethanol drinking depending on history of intake and sex

Authors: *B. PIRINO[1], B. A. CARPENTER[1], G. R. CURTIS[1], A. T. GARGUILO[1], A. N. KARKHANIS[2], J. R. BARSON[1];
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Abstract: The kappa-opioid receptor (KOR) has been investigated as a potential therapeutic target for alcohol use disorder, but the role of the KOR in the nucleus accumbens (NAc) shell, a major reward region, remains unclear. We have previously shown that activation of the KOR in the rostral NAc shell suppresses dopamine release, with no differences detected in males compared to females. Here, we sought to elucidate the effect of KORs in the rostral NAc shell on ethanol drinking, and determine if these effects varied by sex. First, we trained adult male and female Long-Evans rats to drink 20% v/v ethanol under the intermittent access two-bottle-choice paradigm. We then bilaterally microinjected them, prior to daily ethanol access, in the rostral NAc shell with the selective KOR agonist U-50,488H (0.8 or 8.0 nmol) or saline vehicle (0.3 µl), using a within-subject Latin-square design. In male rats, stimulation in the rostral shell significantly decreased ethanol drinking ($p < 0.05$), with effects evident during the first half-hour of daily access, when intake typically is highest. In female rats, however, while KOR activation tended to suppress ethanol drinking in those with a history of lower-level 24-hour intake, it instead significantly enhanced drinking in those with a history of higher-level intake ($p < 0.05$). Conversely, when we injected the long-acting KOR antagonist nor-binaltorphimine (18 mmol) into the rostral NAc shell of ethanol-drinking female rats, we found, 24 hours later, that those with lower-level drinking now showed significantly enhanced ethanol intake, confirming that these effects were physiologically relevant. When we trained a separate group of adult male Long-Evans rats to drink 2.5% w/v sucrose under the same intermittent access paradigm and then microinjected them in the rostral NAc shell with U-50,488H (0.8 or 8.0 nmol) or saline vehicle (0.3 µl), we found no change in sucrose drinking, suggesting that the effects of KOR stimulation on ethanol drinking are substance-specific. These results demonstrate that KOR function in the rostral shell can affect ethanol, but not sucrose, drinking, and that this effect is dependent on sex and history of ethanol intake. Thus, while the KOR represents a promising target for pharmacotherapy to treat alcohol use disorder, such an approach may be more appropriate for specific populations and under specific conditions.

Disclosures: B. Pirino: None. **B.A. Carpenter:** None. **G.R. Curtis:** None. **A.T. Garguilo:** None. **A.N. Karkhanis:** None. **J.R. Barson:** None.

Poster

P740. Alcohol: Circuitry and Neurophysiology of Addiction III

Location: Virtual Only

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Program #/Poster #: P740.07

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH/NIAAA grant AA026078
Washington State University Alcohol & Drug Abuse Research Program grant

Title: High and Low Alcohol Consuming Rodent Genotypes Exhibit Divergent Responses to 10mM Alcohol on Cerebellar Processing, Cerebellar Output to VTA, and Cerebellar Dependent Behavior

Authors: *D. J. ROSSI[1], C. M. ERIKSON[1], K. T. DOUGLAS[1], T. O. THUET[1], N. A. MCLEAN[1], B. D. RICHARDSON[2];
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Abstract: Identifying neural processing targets of low concentrations of alcohol ([EtOH]; 10mM, the blood concentration achieved by an adult human consuming two standard units) is critical for understanding initial psychological reactions to recreational EtOH consumption, for which individual variation influences risk for developing an alcohol use disorder. Emerging evidence has revealed that the cerebellum, traditionally associated solely with motor coordination, is centrally involved in a variety of cognitive processes and maladies including EtOH abuse. We used *ex vivo* patch-clamp and *in vivo* single unit recording, viral tract-tracing, and behavioral assays in high and low EtOH consuming rodent genotypes to determine how 1) molecular/cellular actions of 10mM EtOH vary across rodents with divergent EtOH consumption genotypes, and 2) how such differential actions affect cerebellar neural processing and associated behavioral responses to EtOH. In low EtOH consuming rodent genotypes (DBA/2 mice [D2], C57BL/6N mice [B6N], and Sprague Dawley rats [SDRs]), 10mM EtOH significantly enhances GABAergic inhibition of cerebellar granule cells (GCs), but suppresses such inhibition in high EtOH consuming C57BL/6J (B6J) mice and prairie voles (PVs). To determine how such differences in EtOH's action on GCs affects signal processing through the cerebellar cortex, we recorded action potential output from cerebellar Purkinje cells (PCs; sole output of cerebellar cortex) in slices of cerebellum from 3 genotypes, and *in vivo* in awake B6J mice. In slice experiments, 10mM EtOH did not affect basal PC firing in any genotype, but it selectively increased afferent-evoked PC action potential output in B6J mice. Similarly, with *in vivo* recordings, 10mM EtOH increased sensory/motor-evoked PC output without affecting basal firing rate in B6J mice. To determine how such enhanced afferent-evoked transmission through the cerebellar cortex in B6J mice might interact with more traditional reward circuitry, we injected virally driven channel rhodopsin into the cerebellar dentate nucleus, and found functional direct excitatory (glutamatergic) synaptic connections to neurons within the VTA. In

behavioral studies of EtOH-induced motor impairment, IP injections of EtOH that led to BECs of ~10mM disrupted performance on the rotorod in SD rats, but had no impact in B6J or D2 mice. Moreover, differences in EtOH consumption between genetically similar B6J and B6N mice were not due to differences in taste perception, suggesting a post consumptive, neurological underpinning. Our data identify genotype selective actions of 10mM EtOH in the cerebellum that may influence EtOH consumption.

Disclosures: D.J. Rossi: None. **C.M. Erikson:** None. **K.T. Douglas:** None. **T.O. Thuet:** None. **N.A. McLean:** None. **B.D. Richardson:** None.

Poster

P740. Alcohol: Circuitry and Neurophysiology of Addiction III

Location: Virtual Only

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Program #/Poster #: P740.08

Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA R21AA028088-01 (to NAC)
Brain and Behavior NARSAD Young Investigator Award (to NAC)
Binghamton University Developmental Exposure to Alcohol Research Center P50 SubAward (to NAC)
T32GM108563 (to AS)

Title: Adolescent binge drinking alters a key peptidergic population in the cortex

Authors: *A. SICHER, W. STARNES, N. CROWLEY;
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Abstract: Somatostatin (SST) is a neuropeptide expressed throughout the brain, and is known for its role in multiple psychiatric disorders, but its role in alcohol use disorder has been under-investigated. Ongoing work from our lab has shown key alterations in prelimbic (PL) cortical SST neurons following adulthood exposure to multiple models of alcohol, but little research exists probing SST peptide signaling and neurons during adolescent binge drinking. Multiple lines of evidence point to alcohol-induced alterations of GABAergic and glutamatergic neurons within the cortex following adolescent models of exposure. Here, we use a combination of behavioral, electrophysiological, and optogenetic techniques to elucidate how adolescent binge drinking in the drinking-in-the-dark (DID) paradigm alters SST neuronal properties in the PL cortex. Male and female SST-IRES-Cre x Ai9 mice underwent a modified adolescent version of the DID paradigm of voluntary binge ethanol consumption. Mice were provided access to alcohol throughout the adolescent period (post-natal day 28-60). During this adolescent exposure, mice were provided 20% ethanol weekly for 3 x 2 hr exposures and 1 x 4 hr exposure, 3 hr into the dark cycle. 24 hr after the final ethanol exposure, mice were sacrificed for

electrophysiology, optogenetic manipulations, and other experiments. SST neurons were patched using membrane characteristics and the presence of fluorescence, while pyramidal neurons were identified based on gross morphology and membrane characteristics (cell capacitance and membrane resistance). PL cortical SST neurons in both male and female mice displayed altered intrinsic excitability following alcohol - modifications that were opposite of those seen with adulthood DID exposure. We also demonstrate complementary changes in pyramidal neurons in the same mice. Further experiments demonstrated the time course of adolescent-DID modifications to PL SST neurons (I.e., in acute withdrawal and later in adulthood). Ongoing work will explore the role of SST peptide transmission in this adolescent DID-induced modification of PL SST intrinsic excitability.

Disclosures: A. Sicher: None. **W. Starnes:** None. **N. Crowley:** None.

Poster

P740. Alcohol: Circuitry and Neurophysiology of Addiction III

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Program #/Poster #: P740.09

Topic: G.09. Drugs of Abuse and Addiction

Support: R01 DA034696
R01 AA027544
NIDA/P30 Center of Excellence (1P30 DA048742-01A1)

Title: Deficits in GABA(A) signaling following withdrawal from chronic ethanol exposure in VTA GABA neurons

Authors: *E. H. MITTEN[1], E. MARRON[2], B. HAIDER[3], K. D. WICKMAN[2];
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Abstract: Chronic ethanol exposure has been shown to produce a variety of molecular adaptations within the ventral tegmental area (VTA), with investigations largely focusing on VTA dopamine (DA) neurons. VTA gamma-aminobutyric acid (GABA) neurons are the second largest neuron population (~35%) of the VTA. VTA GABA neurons regulate the excitability of VTA DA neurons as well as other neuronal populations through local and long range projections, respectively. Baseline firing rates of VTA GABA neurons are higher in acute withdrawal following chronic ethanol exposure in vivo. While this has been shown to be due in part to increased glutamate neurotransmission, adaptations to inhibitory signaling contributing to this effect have yet to be fully elucidated. To investigate possible deficits in inhibitory control of VTA GABA neurons, male and female GAD67-eGFP mice were subjected to 3-4 weeks of chronic intermittent ethanol vapor exposure, with subsequent slice electrophysiological

experiments targeting VTA GABA (GFP-positive) neurons performed 1-7 days following the final day of exposure. Animals exposed to air were used as controls. All data were separated by sex and analyzed by two-way ANOVA to assess for sex differences. If no sex differences were detected, data was pooled and analyzed using a Kolmogorov-Smirnov test or Welch's t-test, as appropriate. We found that chronic ethanol exposure suppressed the frequency ($p = < 0.0001$) and amplitude ($p = 0.0375$) of spontaneous GABA(A) receptor-dependent inhibitory postsynaptic currents (sIPSCs) in VTA GABA neurons ($n = 13\text{-}14$). To determine whether these ethanol-induced adaptations reflected postsynaptic GABA(A) receptor density or efficacy, we next measured somatodendritic currents induced by rapid application of the GABA(A) receptor agonist muscimol. Preliminary data show that muscimol-induced currents are significantly reduced in VTA GABA neurons, suggesting that GABA(A) receptor signaling in VTA GABA neurons is significantly reduced following repeated ethanol exposure. Ongoing investigations are directed at identifying relevant GABA(A) receptor subtypes in VTA GABA neurons impacted by repeated ethanol exposure, presynaptic contributions that would produce a reduction in sIPSC frequency, and the behavioral relevance of this form of ethanol-induced neuroplasticity.

Disclosures: E.H. Mitten: None. **E. Marron:** None. **B. Haider:** None. **K.D. Wickman:** None.

Poster

P820. Cortical Networks II

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P820.01

Topic: H.08. Learning and Memory

Support: University of Connecticut Institute of Brain and Behavioral Sciences (IBACS)
Murine Behavioral Neurogentics Facility (MBNF)

Title: A novel assessment of rule-learning in mice using operant touch screen boxes

Authors: *R. CHASSE[1,2], P. A. PERRINO[3], R. H. FITCH[1,2];
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Abstract: Historically, the development of valid and reliable methods for assessing higher order cognitive ability (e.g., rule learning and transfer) has been tricky in rodent models. One barrier is verifying that subjects are actually using cognitive processes assumed by investigators, versus unexpected approaches to solve the task. To date, limited evidence supports the existence of higher cognitive abilities such as rule generation and complex decision making in mice, rats, and rabbits. Although rats have shown marginal evidence of being able to learn and transfer information from one set to another (e.g., attentional set shifting), mice have shown limited

abilities in this domain. Yet the exploding use of engineered mouse models to study genetic disorders characterized by cognitive disruption would benefit tremendously from the inclusion of such a task in mouse phenotyping batteries. For the current experiment, we sought to develop a task that would require mice to learn a visual discrimination task of increasing complexity, following fixed parameters or "rules." Mice would then be asked to apply the remembered rules to a novel (new) image set. Twenty-eight (28) wild-type male c57 mice performed the initial visual discrimination, which was conducted in Bussey-Saksida touchscreen operant boxes. We used 2 different image sets, counterbalanced for the image set each group received first. Results showed that mice were able to quickly learn the first image set, and could learn the "rule" based on performance curves that were significantly better than initial learning when image sets became more complex. They could also remember this information many weeks later. However, the mice appeared unable to transfer the rule to a *new* set of stimuli. Specifically, they showed visual discrimination curves for the new image set that was no better than the first (naïve) round of learning. We continue to revise this task to optimize stimulus parameters and maximize task sensitivity, hoping to better tap these important cognitive measures in mice.

Disclosures: R. Chasse: None. **P.A. Perrino:** None. **R.H. Fitch:** None.

Poster

P820. Cortical Networks II

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P820.02

Topic: H.08. Learning and Memory

Support: MH109464

Title: Menstrual Cycle Influences on the Intrinsic Network Structure of the Brain

Authors: A. GIANCOLA[1], N. MCLACHLAN[1], B. C. DICKERSON[2], L. F. BARRETT[3], E. K. TOWLSON[4], *J. ANDREANO[1];
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Abstract: Growing research indicates that changes in ovarian hormone levels over the menstrual cycle and the lifespan can have significant influences on brain function. Prior studies have indicated ovarian effects on connectivity, particularly between nodes of the default mode network (DMN) and salience network (SN), two intrinsic networks known to play an important role in learning, memory, and affect, leading us to hypothesize that cyclic changes in connectivity may lead to ‘windows of vulnerability’ to affective disorder (1). No study to date, however, has examined the possibility that the organization of resting networks themselves may

change in response to ovarian signals. Here we present data from 100 resting-state fMRI scans in 22 women taken at three hormonally distinct phases of their menstrual cycle: (i) early follicular (EF), when ovarian hormone levels are low; (ii) late follicular (LF), when estrogen levels reach their peak; and (iii) mid-luteal (ML), when both estrogen and progesterone are elevated. We constructed whole-brain functional connectomes for each participant at each of the three menstrual phases by calculating the Pearson's correlation coefficient between regional fMRI timeseries. We then identified 6 communities, or subnetworks, within the brain via the Louvain algorithm (2) applied to averaged connectomes for EF, LF, and ML groups. The resulting community structure of the brain was then compared between menstrual phases. Specifically, we investigated the changing assignment of nodes to subnetworks, and the connectivity distributions within and between the subnetworks. Differences were quantified using the Mann-Whitney Test.The results indicated significant changes in intrinsic network structure between phases. Notably, our findings indicate a stronger functional fractionation of the midline and temporal components of DMN during the LF phase. Additionally, the mid-cingulate cortex, a network hub engaged by difficult tasks, shifted its connections from the DMN and SN in EF and ML to the dorsal attention network in the LF phase. These changes in network organization accompanied phase-related differences in cognition and affect that were similarly centered on the LF phase. Potential links between connectivity changes and sex/phase differences in affective disorder, as well as their implications for women's health, will be considered. 1. Andreano, JM. et al. "Hormonal cycles, brain network connectivity, and windows of vulnerability to affective disorder" TINS (2018) 2.Blondel, Vincent D., et al. "Fast unfolding of communities in large networks." Journal of statistical mechanics: theory and experiment 2008.10 (2008): P10008.

Disclosures: A. Giancola: None. **N. McLachlan:** None. **B.C. Dickerson:** None. **L.F. Barrett:** None. **E.K. Towlson:** None. **J. Andreano:** None.

Poster

P820. Cortical Networks II

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Program #/Poster #: P820.03

Topic: H.08. Learning and Memory

Support: Howard Hughes Medical Institute
NIH Grant 5F32MH122147

Title: Cortical Amygdala Nuclei Involvement in Olfactory Fear Memory Processing

Authors: *C. DE SOLIS, A. ASOK, J. LOPEZ-ROJAS, P. CHEN, E. R. KANDEL; Columbia Univ., New York, NY

Abstract: The memories of our lives, good and bad, are constructed from our senses. In the past decades, we have learned a great deal about the fascinating neurobiological mechanisms that enable sensory information to be woven into a memory. In addition to visual, gustatory, tactile, and auditory information, our representation of the world and how we behave in it is heavily influenced by our ability to detect odors. For example, the smell of burning rubber, blood and gasoline can serve as potent retrieval cues, particularly among individuals suffering from fear- and anxiety-related psychiatric disorders. Yet, our knowledge of how learned olfactory information is encoded and updated is relatively incomplete, especially regarding aversive memories. Here, we examine the involvement of amygdala cortical nuclei in the formation of olfactory fear memories. We find that a subdivision of these nuclei show increased activation in response to olfactory fear conditioning, as detected by levels of intracellular calcium and immediate early gene expression. We then silenced this region during classical fear conditioning to an olfactory, auditory or visual conditioned stimulus, however, while deficits in long-term olfactory memory retrieval were apparent, visual and auditory retrieval was left intact. We then performed neuronal tracing experiments to identify the cortical nucleus projections to the medial prefrontal cortex (mPFC). Using a dual-viral approach to deliver chemogenetic and optogenetic tools to this discrete circuit, we determined that amygdala cortical nuclei neurons projecting to the mPFC play a role in the acquisition of odor fear memories.

Disclosures: C. de Solis: None. **A. Asok:** None. **J. Lopez-Rojas:** None. **P. Chen:** None. **E.R. Kandel:** None.

Poster

P820. Cortical Networks II

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Program #/Poster #: P820.04

Topic: H.08. Learning and Memory

Support: ERC No 819526
ESRC ES/J500173/1
ESRC ES/V012444/1

Title: Repeated & predictable information dominates the neural representation of complex events

Authors: *P. RAYKOV[1], K. BROMIS[2], L. WICKENS[2], W. ROSEBOOM[2], C. M. BIRD[3]; [1]Sussex Univ., Brighton, United Kingdom; [2]Univ. of Sussex, Brighton, United Kingdom; [3]Sch. of Psychology, Univ. of Sussex, Falmer, Brighton, United Kingdom

Abstract: An episodic memory is specific to an event that occurred at a particular time and place. However, the elements that comprise the event – the location, the people present, their

actions and goals – might be shared with numerous other similar events. Does the brain preferentially represent certain elements of a remembered event? If so, does it prioritise the familiar and predictable elements that are shared across similar events, or the novel elements that define a specific event? We addressed this question by using a novel experimental paradigm combined with fMRI. Multiple events were created involving conversations between two individuals, using the format of a television chat show. Chat show "hosts" occurred predictably in multiple events, whereas the "guests" were unique to only one event. Before learning the conversations, participants were scanned while viewing images or names of the (famous) individuals to-be-used in the study, in order to obtain person-specific activity patterns. After learning all the conversations over a week, participants were scanned for a second time while they recalled each event multiple times. We found that during recall, person-specific activity patterns within the posterior midline (PM) network were reinstated for the hosts of the shows but not the guests, and that reinstatement of the hosts was significantly stronger than reinstatement of the guests. These findings demonstrate that the familiar and predictable elements of an event are represented in the brain more robustly than the novel, yet event-defining, elements.

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Poster

P820. Cortical Networks II

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Program #/Poster #: P820.05

Topic: H.08. Learning and Memory

Support: R01MH110594
R01MH116937

Title: High channel-count neural recordings in monkeys reveal the diverse underpinnings of novelty detection across brain circuits

Authors: *K. ZHANG[1], E. S. BROMBERG-MARTIN[5], F. SOGUKPINAR[2], K. KOCHER[5], I. E. MONOSOV[5,1,2,3,4];
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Abstract: Humans and other primates learn by exploring novel objects, but the neuronal mechanisms of novelty detection are unclear. It is well known that neurons in many brain areas respond differently to novel versus familiar objects (Ranganath et al., 2003). Several classes of theories propose that these novelty responses are derived from computations of sensory surprise

(Kumaran, 2007) or recency (Vogels, 2015). However, these mechanisms could be interdependent. Novelty responses may arise from computations of both sensory surprise and recency, or neither. Also, novel objects mediate behavior on different timescales, flexibly depending on context. But whether novelty-sensitive neurons display heterogenous timescales of novelty-to-familiarity learning to support such behavioral flexibility remains unclear. To study these issues, we recorded thousands of neurons across temporal cortex, amygdala, hippocampus, basal ganglia, and the prefrontal cortices while two monkeys participated in a behavioral procedure that tested the relationship of novelty, sensory surprise, and recency. During neural recording, monkeys viewed sequences of visual fractal objects. These fractals could be novel or familiar, more or less recent, and more or less surprising. Across many brain areas, we found that neurons selectively responding to novel objects also tended to respond to sensory-surprising objects and non-recent objects in the same direction. Among novelty-excited neurons, there were significant positive correlations between neurons' novelty sensitivity and their sensory surprise and recency sensitivities. Moreover, novelty neurons also showed spatial clustering, with particular enrichment in the anterior ventral medial temporal regions. In general, brain areas with a higher percentage of novelty responsive neurons tended to have higher percentages of sensory surprise responsive and recency responsive neurons. Control experiments indicated that these response patterns were not simply due to other aspects of the behavioral procedure such as general arousal or reward. Finally, we studied neuronal novelty-to-familiarity transformations during learning across many days from repeated exposure to novel fractals. We found a wide spectrum of timescales in neurons' learning rates and forgetting rates across multiple days that is well suited to support flexible adaptive behavior to novel objects. Our findings show that novelty detection arises on multiple timescales due to diverse but related computations of sensory surprise and recency, and shed light on the logic and computational underpinnings of novelty detection in the primate brain.

Disclosures: K. Zhang: None. **E.S. Bromberg-Martin:** None. **F. Sogukpinar:** None. **K. Kocher:** None. **I.E. Monosov:** None.

Poster

P820. Cortical Networks II

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Program #/Poster #: P820.06

Topic: H.08. Learning and Memory

Support: ERC Advanced Grant ERC-2017-ADG, FOUNDCOG, 787981

Title: Are intrinsic timescales innate or emergent? Intrinsic timescales by brain region in human neonates and adults.

Authors: A. TRUZZI[1], *R. CUSACK[2];
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Abstract: In adults, each brain region has a characteristic intrinsic timescale over which it integrates information. Electrophysiology in macaques and EEG/MRI in humans have shown that these timescales follow a gradient from shorter in primary sensory regions to longer in higher-order regions. Disruption to intrinsic timescales has been associated with cognitive impairments and can predict the severity of symptoms in Autism Spectrum Disorders. It has not been established how timescales develop in infancy. The characteristic timescales of brain regions might be an innate property that guides subsequent learning. Or, the timescales might emerge in the course of learning, with more persistent representations emerging as the statistics of the environment are learned. Here, we characterised the timescales using resting-state fMRI in low-movement neonates (FD < 0.25mm) from the developing human connectome project (dHCP, N_infant = 267) and low-movement adults from the CamCAN project (N_adult = 252). Timescales were calculated for 400 regions-of-interest (ROIs) using an autoregressive model. The order of the model was selected on a subset of the dHCP data and cross-validated on an independent subset. Timescales across ROIs in the two independent dHCP groups were strongly correlated (Spearman's $r = 0.77$). In some brain systems, the timescales in a neonate's brain follow a similar gradient to the one found in adults, from shorter in primary sensory regions to longer in higher-order regions. However, unimodal and transmodal regions showed different patterns in infants and adults (Kruskal-Wallis $H = 395.5$, $p < 0.001$). No significant difference was found between unimodal and transmodal networks in infants, whereas a significant difference between the two types of networks was found in adults (Mann-Whitney $U = 14797$, $p < 0.05$, $d = 0.36$). Moreover, infants showed overall longer timescales compared to adults, both in unimodal (dHCP Group1: Mann-Whitney $U = 227$, $p < 0.001$, $d = 3.18$; dHCP Group2: Mann-Whitney $U = 238$, $p < 0.001$, $d = 3.18$) and transmodal regions (dHCP Group 1: Mann-Whitney $U = 10462$, $p < 0.001$, $d = 0.58$; dHCP Group2: Mann-Whitney $U = 10605$, $p < 0.001$, $d = 0.53$). Differences in the timescales between brain regions are therefore present already at birth and are likely to shape learning. However, infants' primary sensory and motor regions show a slower intrinsic timescale at birth compared to adults. Since slower neural processes allow the integration of incoming information across longer timescales it might help the human infants to "take it slow" and to create more regularised, and holistic representations of the input which, in turn, could improve later memory and decision making.

Disclosures: A. Truzzi: None. **R. Cusack:** None.

Poster

P820. Cortical Networks II

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P820.07

Topic: H.08. Learning and Memory

Support: NSERC

Title: Disrupting GABAergic transmission impairs category recognition on a mouse object category recognition task

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Abstract: Recognizing visually distinct objects as belonging to the same category - and distinguishing between different categories - is necessary for producing object-appropriate behavioural responses. Objects from a familiar category may be recognized with the use of generalized category representations which may be refined through exposure to various category exemplars. Presumably, to refine category representations, extraneous object features must be inhibited, to allow distinction between category representations and to enable recognition of a novel object from a familiar category. Parvalbumin-containing GABAergic interneurons (PVINs) has been implicated in visual stimulus learning, including the enhancement and inhibition of relevant and irrelevant stimuli, respectively. Furthermore, PVINs has been identified as providing feedforward inhibitory control in the perirhinal cortex (PRh), an area which is necessary for object recognition and object feature-binding. To determine the role of GABAergic transmission in the refinement of object category representations, male C57/BL6 mice were administered a $GABA_A$ receptor antagonist (bicuculline; 0.5mg/kg, i.p.) prior to exposure to various category exemplars and were subsequently tested on a mouse object category recognition (OCR) task. Mice require previous exposure to category exemplars to perform the OCR task with long retention delays, such as 1-h. Mice administered bicuculine prior to exposure to these exemplars were not able to discriminate between a novel and familiar category object after a 1-h delay on the OCR task. Next, to specifically examine the role of GABAergic PVINs in the PRh in the formation of generalized category representations, we infused male PV^{cre} mice intracranially with adeno-associated virus containing inhibitory designer receptors exclusively activated by designer drugs (DREADDs) to control the activity of PVINs in the PRh. The DREADD agonist 21 (11-(1-piperazinyl)-5H-dibenzo[b,e][1,4]diazepine; C21) was administered systemically (i.p., 1mg/kg) prior to exposure to object category exemplars. Mice administered C21 prior to pre-exposure sessions also failed to discriminate between a novel and familiar category object when tested subsequently on the OCR task with a retention delay of 1-h. These findings suggest that GABAergic transmission, and specifically PVIN activity in the PRh, may play an essential role in the refinement of object category representations, and further our understanding of the processes that facilitate category learning and recognition.

Disclosures: H.A. Collett: None. **S.D. Creighton:** None. **J.L. Fournier:** None. **R.A. Pandit:** None. **K.H. Jardine:** None. **B.D. Winters:** None.

Poster

P820. Cortical Networks II

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P820.08

Topic: H.08. Learning and Memory

Support: R00DC015014
AFAR Young Investigator Award

Title: Revealing hidden sensorimotor memories in mice with AD-relevant pathology

Authors: S. MOORE[1], A. WANG[1], J. LAWLOR[1], K. FOGELSON[1,2], ***A. SANTI**[1], K. KUCHIBHOTLA[1,3];
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Abstract: Memories must be accessible for them to be useful. Alzheimer's disease (AD) is a progressive form of dementia in which cognitive capacities slowly deteriorate due to underlying neurodegeneration. Interestingly, anecdotal observations have demonstrated that Alzheimer's patients can exhibit cognitive fluctuations during all stages of the disease. In particular, it is thought that contextual factors are critical for unlocking these hidden memories. To date, however, exploration of the neural basis of cognitive fluctuations has been hampered due to the lack of a behavioral approach in mouse models to dissociate memories from contextual performance. Our previous work demonstrated that interleaving 'reinforced' trials with trials without reinforcement ('probe' trials) in an auditory go/no-go discrimination task, allows us to distinguish between acquired sensorimotor memories and their contextual expression. Here, we used this approach, together with two-photon calcium imaging on behaving AD-relevant mice (APP/PS1+), to determine whether amyloid accumulation impacts underlying sensorimotor memories (measured using 'probe' trials) and/or contextual-performance (measured using 'reinforced' trials) in an age dependent manner. Importantly, peripheral auditory function, measured using the threshold for detecting an auditory brainstem response, was similar between WT and APP/PS1+ mice. We found that while contextual-performance is significantly impaired in young adult APP/PS1+ mice compared to age-matched controls, these animals show only minor impairments in the underlying sensorimotor memories. However, middle aged APP/PS1+ mice show deficits in both domains. The impairment found in the young adults was accompanied by a reduction in stimulus selectivity in the auditory cortex of APP/PS1+ mice that was particularly apparent in reinforced trials. Ongoing analyses aim to identify whether this impairment is cortex-wide or is concentrated near Aβ plaques. Finally, these effects were recapitulated by using a reinforcement learning model that accounts for changes in contextual signals. The main network model parameters affected between the control and the APP/PS1+ mice were those governing contextual scaling and behavioral inhibition. These results suggest

that Aβ deposition impacts circuits involved in contextual computations before those involved in acquiring knowledge.

Disclosures: S. Moore: None. **A. Wang:** None. **J. Lawlor:** None. **K. Fogelson:** None. **A. Santi:** None. **K. Kuchibhotla:** None.

Poster

P820. Cortical Networks II

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Program #/Poster #: P820.10

Topic: H.08. Learning and Memory

Support: NEI R01EY031455
ONR N00014-20-1-2002
NIH/NIA RF1 AG029577

Title: Model connectivity: a new approach to functional connectivity that leverages the power of voxelwise encoding models to eliminate confounding noise and increase interpretability

Authors: *E. X. MESCHKE[1], M. VISCONTI DI OLEGGIO CASTELLO[2], J. L. GALLANT[3]; [1]Univ. Of California, Berkeley Neurosci. Grad. Program, Berkeley, CA; [2]Univ. Of California, Berkeley, Berkeley, CA; [3]Univ. of California Berkeley, Berkeley, CA

Abstract: Complex cognitive functions are subserved by anatomically coupled networks of brain areas. The most common method for identifying these networks in the human brain is to use neuroimaging data to estimate functional connectivity (FC). FC is defined as the correlation of BOLD signals over time between spatially distinct brain areas. Although FC studies are popular, they suffer from two fundamental flaws. First, because FC correlates BOLD signals directly, true signal correlations that reflect underlying anatomical connectivity cannot be separated from noise correlations that contaminate all functional MRI studies. This makes it difficult to use FC to draw valid conclusions about the coupling of brain areas. Second, it is difficult to determine what information is represented in networks identified with FC without additional experiments.
To overcome both flaws, we developed model connectivity (MC). MC builds on the voxelwise encoding model (VM) framework. In VM, the activity in each voxel is modeled in terms of stimulus- or task-related features. Regularized regression is used to predict brain activity from those features, and model prediction accuracy is estimated on a separate test dataset. This produces model weights that quantify how voxels represent stimulus-related features. Using separate data sets to fit models and to evaluate performance ensures that model weights are based on predictable signals, not noise. MC uses the correlations between model weights to recover functional networks that are not contaminated by noise and which have a clear functional

assignment.
To compare MC to FC, we applied both methods to an fMRI dataset in which participants listened to narrative stories (Huth et al., 2016). For MC, we used VM to predict brain activity from the semantic content of the stories, and we used hierarchical clustering to identify networks based on model weights. For FC, we applied hierarchical clustering to the pre-processed BOLD activity. We make three observations about the networks produced by these two methods. First, we find that MC excludes noisy voxels that constitute a large part of networks recovered by FC. Furthermore, networks recovered by MC are more spatially specific than those recovered by FC. Finally, because the MC networks are based on voxelwise encoding model weights, they are more interpretable than the FC networks.
We conclude that MC is a powerful new method for identifying functional networks in the human brain. This approach does not suffer from noise confounds that contaminate conventional FC studies and produces networks with clear functional assignments.

Disclosures: E.X. Meschke: None. **M. Visconti di Oleggio Castello:** None. **J.L. Gallant:** None.

Poster

P831. Hippocampal Circuits and Memory

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Program #/Poster #: P831.01

Topic: H.08. Learning and Memory

Support: NIH Grant F31 AG067718-02
NIH Grant R01 MH111773-03S1

Title: Basal forebrain cholinergic circuit orchestrates adult hippocampal neurogenic niche to regulate neural stem cells

Authors: *L. J. QUINTANILLA[1,2], J. M. SIMON[3,4,5], Y. SU[6], Y. LUO[2], B. ARISCAN[2], D. TART[2], Y. LI[2], J. SONG[2,3];
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Abstract: The adult mammalian hippocampus contains endogenous neural stem cells (NSCs) that provide a self-renewable source for continuous replenishment of new neurons throughout life. The NSCs reside in a specialized niche within the dentate gyrus (DG) that comprises diverse populations of niche cells. Our recent studies demonstrate that these local niche cells can regulate the activation/quiescent state of NSCs and subsequent production of newborn progeny. Whether there is a master regulator that can orchestrate the actions of these local niche cells to

regulate NSCs remains unknown. Here we identified a basal forebrain cholinergic circuit projected from Diagonal Band of Broca (DB) to the DG, which exerts broad actions on multiple types of local niche cells. Functionally, activation of the DB-DG cholinergic circuit increased proliferation of NSCs and production of newborn progeny. Interestingly, the dominant signaling onto NSCs is glutamatergic upon stimulation of the DB-DG circuit, suggesting that cholinergic signaling acts upon intermediary niche cells capable of releasing glutamate to regulate NSCs. To further address cholinergic circuit induced effects in a cell-type specific manner, we performed single nuclei RNA-Seq of the DG upon stimulation of the DB-DG cholinergic circuit. Notably, we identified broad distribution of differentially expressed genes (DEGs) across multiple types of niche cells, including various types of interneurons, mossy cells (MCs), granule cells (GCs), and astrocytes. The common DEGs associated with these cell clusters were involved in trans-synaptic signaling, membrane ion transport, and synapse formation, suggesting DB cholinergic activation of these cell clusters. Importantly, these niche cell types, upon activation, are capable regulating NSCs directly or indirectly through glutamatergic signaling. Consistent with cholinergic activity dependent regulation of NSCs through glutamatergic signaling, we identified the DEGs in NSCs involved in NMDA glutamate receptor activity, voltage gated ion channels, and calcium ion transmembrane activity. Together, our results support that the basal forebrain cholinergic circuit acts as a master regulator to orchestrate neurogenic niche and regulate NSCs.

Disclosures: L.J. Quintanilla: None. **J.M. Simon:** None. **Y. Su:** None. **Y. Luo:** None. **B. Ariscan:** None. **D. Tart:** None. **Y. Li:** None. **J. Song:** None.

Poster

P831. Hippocampal Circuits and Memory

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Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P831.02

Topic: H.08. Learning and Memory

Support: HHMI

Title: Cellular mechanisms of context dependent feature selectivity in hippocampal CA3: inputs and plasticity

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Abstract: Hippocampal place cells are believed to encode “where” in spatial navigation and episodic memory. Place cells are not passively driven by the specific content features, but fire in a context dependent manner. That is, the same feature in completely different environments will produce disparate place cells firing, or trigger distinct memory traces. However, how environmental context determines the place field and drives place field remapping is still an open

question. Here I am trying to uncover the source of context dependent remapping that is the strongest in hippocampal CA3. I trained head-fixed mice running on a linear treadmill with different feature cues. The corresponding subthreshold membrane potentials and action potentials have been recorded and measured through whole-cell intracellular voltage recordings. I firstly examined the BTSP (behavioral time scale plasticity) in CA3, and found that the dendritic calcium plateau potentials, rather than spike trains, could produce a large potentiation in CA3 as in CA1, but with a completely different plasticity kernel. The kernel in CA3 is symmetric compared to the asymmetric one in CA1, which endue CA3 to produce a current map relative to the predictive map in CA1. These two maps together could provide the current and future information to downstream. Meanwhile, I found that CA3 cells activity could be separated into 5 categories during the task, which may contribute to build an attractor network within CA3 to generate a sequence covering the entire "space" of the task. And the mismatch of sequence and spatial cue can well explain different remapping behavior of CA3 place cells after shifting the reward location. Thus, we think the role of CA3 attractor network is to generate a specific sequence/trajectory for different contexts. Next, I am trying to optogenetically manipulate CA3 and its upstream activity, to examine their role in building the CA3 attractor network.

Disclosures: Y. Li: None.

Poster

P831. Hippocampal Circuits and Memory

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P831.03

Topic: H.09. Spatial Navigation

Support: NSF Grant NeuroNex #1707287
NIH Grant R01NS121919

Title: Structural plasticity of apical dendritic spines in hippocampal CA1 is modulated by sex-specific steroid hormones

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Abstract: Dendritic spines are highly dynamic and motile structures that serve as the postsynaptic sites of excitatory synapses in the hippocampus. Previous *in vitro* studies suggest that the structural and functional plasticity of dendritic spines may be tied to sex-specific steroid

hormones such as estradiol and progesterone, the concentrations of which fluctuate over the course of the 4-5 day murine estrous cycle. However, the *in vivo* turnover dynamics of apical dendritic spines in hippocampal pyramidal cells remain unknown. Estrous-dependent fluctuations in dendritic spine density were consistently observed over consecutive cycles using our recently developed chronically implanted hippocampal microperiscope in awake mice. When identifying estrous stage, human error was greatly reduced by implementing EstrousNet, a novel unsupervised machine learning approach that provides unbiased stage classification. Using these novel techniques, we found that apical dendritic spines in female hippocampal CA1 experience a 15.3% increase in density in late proestrous, followed by a 20.6% decrease in spine density in early estrous. In both sexes across 10 days we found only a 23.5% net loss in original spines, indicating that majority of spine turnover takes place within an isolated population of transient spines. Taken together, these findings inform our understanding of structural and functional plasticity in hippocampal pyramidal cells as they relate to sex-specific steroid hormones.

Disclosures: N. Wolcott: None. **W. Redman:** None. **K. Sit:** None. **M. Goard:** None.

Poster

P831. Hippocampal Circuits and Memory

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Program #/Poster #: P831.04

Topic: H.09. Spatial Navigation

Support: NeuroNex Grant 1707287
NIH Grant R01NS121919
Whitehall Foundation Grant
Larry Hillblom foundation Grant

Title: Long-term transverse imaging of the hippocampus with glass microperiscopes

Authors: *W. REDMAN[1], N. WOLCOTT[2], L. MONTELISCIANI[7], G. LUNA[3], T. MARKS[3], K. SIT[4], C.-H. YU[5], S. L. SMITH[5], M. GOARD[6];
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Abstract: The transverse axis of the hippocampus consists of a stereotyped neuronal circuit repeated along the septal-temporal extent of the structure. This circuit contains distinct subfields with well-described connectivity that supports crucial cognitive processes, such as episodic and spatial memory. While extensive research has provided insight into hippocampal function,

existing physiological approaches do not allow for comprehensive measurement of the transverse hippocampal circuit *in vivo*. To address this, we developed an approach for two-photon imaging of the transverse hippocampal plane in awake, behaving mice via chronically implanted glass microperiscopes. This microperiscope allows optical access to all major hippocampal subfields (i.e. CA1-3, and dentate gyrus) in the same animal, as well as to the apical and basal dendrites of the pyramidal neurons. Using this approach, we chronically imaged spines on apical CA1 dendrites and characterized spine turnover across days, finding both transient and stable populations. We then used calcium imaging to quantify the prevalence of place and speed cells across subregions, finding a non-uniform distribution of spatial selectivity along the DG-to-CA1 axis. In particular, we found that cells in dorsal CA3/CA2 had the lowest spatial information, and that cells in proximal CA3 had values that were most similar to DG cells. This approach will extend the existing toolbox for structural and functional measurements of the hippocampal circuitry supporting memory formation.

Disclosures: W. Redman: None. **N. Wolcott:** None. **L. Montelisciani:** None. **G. Luna:** None. **T. Marks:** None. **K. Sit:** None. **C. Yu:** None. **S.L. Smith:** None. **M. Goard:** None.

Poster

P831. Hippocampal Circuits and Memory

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Program #/Poster #: P831.05

Topic: H.04. Executive Functions

Support: NIH Grant MH096274

Title: Novel experience enhances flexible learning by resetting hippocampal-prefrontal circuitry

Authors: *A. PARK[1], A. HARRIS[1], K. MARTYNIUK[1], C.-Y. CHANG[1], A. I. ABBAS[3], D. C. LOWES[2], C. KELLENDONK[4], J. GOGOS[1], J. A. GORDON[5];
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Abstract: Adapting to novel situations is essential for everyday life, and this flexibility is often impaired in psychiatric disorders. Thus, understanding whether and how novelty prepares, or primes, the brain to promote cognitive flexibility has important translational relevance. Here, we show that novel experience, either spatial or social, primes the brain by resetting the ventral hippocampus (vHPC)-medial prefrontal cortex (mPFC) circuit in a dopamine D1-receptor (D1R)-dependent mechanism. This reset boosts adaptation to new rules. Data analyses were blind to the groups of a total of 132 mice (both gender). We trained mice to freely choose either arm of a T-maze for rewards (‘free choice’). Over three days, mice developed a strategy to

simply choose one particular arm (arm bias). Then, in the same maze, the mice underwent 'flexible choice' training, which required them to overcome their arm bias and flexibly choose a goal under new rules (delayed-non-match-to-sample). Mice exposed to a novel arena or a novel mouse 1-hour before training overcame their arm bias more rapidly than mice exposed to familiar conditions. We also performed electrophysiological recordings in the HPC and mPFC as they govern spatial learning. Novelty persistently amplified vHPC theta (4-12 Hz) oscillations at least for 1-hour until the onset of flexible choice task and entrained vHPC neurons to the trough of theta oscillations. Moreover, three independent measures (vHPC activity-triggered mPFC neuronal spiking, the phase-locking of mPFC neurons to vHPC theta oscillations, and optogenetically evoked vHPC-to-mPFC synaptic transmission) indicated that novelty weakened existing vHPC-mPFC connectivity. This weakened connectivity re-strengthened as novelty-exposed mice adapted to the flexible choice task. Machine learning and information theory analyses revealed that novelty reduced adherence to arm bias by vHPC theta entrainment and vHPC-mPFC connectivity weakening. Also, task-specific strategy updating occurred as the weakened connectivity re-strengthened. Conversely, in familiar conditions, vHPC-mPFC connectivity remained stable, and mice adhered to their arm bias. Finally, this effect of novelty was mimicked by the D1R agonist dihydrexidine treatment, while blocked by infusing the D1R antagonist SCH23390 into the vHPC or optogenetic inhibition of novelty-tagged vHPC neurons expressing D1Rs. Hence, novel experience primes the brain by D1R-mediated resetting of vHPC-mPFC circuitry, facilitating new learning. As pathologic vHPC-mPFC connectivity is prominent in many psychiatric disorders, our work provides new mechanistic insight for therapeutic interventions.

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Poster

P831. Hippocampal Circuits and Memory

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P831.06

Topic: H.08. Learning and Memory

Title: Determining the true magnitude of recognition impairments following neurotoxic lesions of the hippocampus in nonhuman primates: A meta-analysis

Authors: *S. J. WATERS[1,2], B. M. BASILE[3], E. A. MURRAY[2];
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Abstract: Studies of localized brain damage provide powerful evidence for the causal link between neural structure and cognitive function. Despite decades of research on memory function following experimentally induced lesions of medial temporal lobe structures, the role of the hippocampus in recognition memory remains disputed, particularly with nonhuman primate subjects. We aim to resolve this dispute by analyzing both individual and aggregate data collected from 14 publications that assess visual item recognition in nonhuman primates with and without selective neurotoxic lesions of the hippocampus. Subgroup and cumulative meta-analyses indicated the overall distributions of recognition performance scores for subjects with and without hippocampal lesions overlap by approximately 74 percent (Hedges' g = -0.66). Paradoxically, a meta-regression that investigated the relation between lesion extent and effect size indicated that more damage to the hippocampus significantly predicted better memory on visual item recognition tasks. Additionally, the impact of methodological differences on effect-size estimates is assessed. We conclude that hippocampal integrity, alone, is not necessary for visual item recognition in nonhuman primates.

Disclosures: S.J. Waters: None. **B.M. Basile:** None. **E.A. Murray:** None.

Poster

P831. Hippocampal Circuits and Memory

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Program #/Poster #: P831.07

Topic: H.08. Learning and Memory

Support: PRE2018-084504
PID2019-110755RB-I00
2002_2020B

Title: Defective memory engram allocation in Down syndrome

Authors: Á. FERNÁNDEZ-BLANCO[1], A. ZAMORA-MORATALLA[1], C. GONZÁLEZ-ARIAS[2], G. PEREA[2], M. DIERSSEN[1];
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Abstract: Cognitive dysfunction in Down syndrome (DS) has an extremely heterogeneous and complex pathophysiology, not yet understood. Technological and conceptual breakthroughs in neuroscience have made possible to visualize and manipulate the neural correlates of memory, leading to the concept of memory engrams. Neuronal and synaptic signaling alterations in DS mouse models, suggest DS as a model of inherently defective engrams. We have investigated engram-specific alterations using engram tagging techniques and chemogenetic intervention in Ts65Dn, a validated DS mouse model. Using immunostaining and recombinant AVV-TRE-tight-

hM3Dq-mCherry, we detected sparser neuronal activation in Ts65Dn 3 month old male mice both after learning and after recall, as shown by the reduced number of cells expressing *c-Fos* and *Arc* immediate early genes in the dentate gyrus. Engram reactivation using chemogenetic intervention with clozapine N-oxide (CNO) did not recover Ts65Dn memory deficits. This would indicate that the trisomic engram is not properly formed, thus preventing the memory to be completely recalled in the future. We conclude that neuronal allocation to an engram is a possible mechanism of engram pathology in DS that would underlie memory deficits.

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Poster

P831. Hippocampal Circuits and Memory

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Program #/Poster #: P831.08

Topic: H.08. Learning and Memory

Support: Departamento de Ciencias de la Salud, Unidad Lerma to KRGR
DGAPA PAPIIT IN210817 to FBR

Title: Environmental enrichment improves memory and synaptic plasticity in cognitively impaired animals due to chronic exposure to a high-fructose and high-fat diet.

Authors: *E. GUTIERREZ-LOPEZ[1], F. BERMUDEZ-RATTONI[1], K. GUZMAN-RAMOS[2]; [1]Dept. de Neurociencias, Inst. De Fisiologia Celular, UNAM, Mexico City, Mexico; [2]Dept. de Ciencias de la Salud, UAM Lerma, Lerma de Villada, Mexico

Abstract: Metabolic deregulations like the excessive accumulation of body fat, hyperglycemia, and overweight are considered risk factors for the development of cognitive and neuronal plasticity impairments. The mechanisms underlying these declines are unknown. However, several studies indicate that chronic exposure to high-calorie diets affects neuronal plasticity and reduces the catecholaminergic levels in hippocampus, these neurotransmitters act as neuromodulators for the formation of declarative memory and plastic changes that underly memory trace formation and maintenance. Increasing catecholaminergic levels with an intra-hippocampal microinjection of nomifensine in rats that were chronically exposed to high-calorie diets recovers memory and synaptic plasticity, showing that the restoration of dopaminergic activity reverse the deterioration of recognition memory and long-term plasticity. However, it is not known whether implementing a non-pharmacological treatment that increases the amount of catecholamines in hippocampus, such as environmental enrichment, could reverse the negative effects of chronic consumption of these diets on synaptic plasticity and memory. To test this hypothesis, a group of male mice were exposed to a high-fructose and high-fat diet for 6 months

and compared to control group of male mice exposed to standard diet during the same period. Behavior was evaluated by object location memory task and neuronal plasticity was evaluated by long-term potentiation in the perforant pathway to dentate gyrus synapsed. The high-fructose and high-fat diet caused an increase in adiposity and glucose intolerance, constituting a model of metabolic deregulation. The behavioral results indicate an impairment of the spatial memory and long-term potentiation shows a deficiency in the synaptic strength in mice with the aforementioned diet. In addition, concomitant 6-month enrichment environment consisting of frequent replacement of a variety of toys in of different sizes, colors, shapes and textures within the home-cage of mice with high-fructose and high-fat diet produced an increase in synaptic strength similar to controls. Likewise, mice with metabolic deregulations exposed to environmental enrichment behaved similarly to controls in spatial recognition memory tests. These results show that the enriched environment can prevent the deterioration of recognition memory and long-term plasticity. These results will help to lay the foundations for the understanding of protective factors and treatment approaches for the deterioration of memory associated with chronic consumption of hypercaloric diets.

Disclosures: E. Gutierrez-Lopez: None. **F. Bermudez-Rattoni:** None. **K. Guzman-Ramos:** None.

Poster

P866. Place Cells I

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Topic: H.09. Spatial Navigation

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NIH R01 MH060013
NIH R01 MH120073
NIH R01 HD101402-02
NIH DP5 OD023106-01
ONR MURI N00014-16-1-2832
ONR MURI N00014-19-1-2571

Title: Physiological and optogenetic assessment of the role of engram ensembles in spatial working memory in mouse dentate gyrus

Authors: *L. K. WILMERDING[1], I. KONDRATYEV[2], S. RAMIREZ[2], M. E. HASSELMO[3]; [1]Boston Univ. Grad. Program For Neurosci., Boston, MA; [3]Psychological and Brain Sci., [2]Boston Univ., Boston, MA

Abstract: The hippocampus has been studied extensively for its role in navigation and episodic memory. To analyze the nature of specific episodic experiences and their role in memory-guided navigation behavior, we examined ensembles of co-active cells, 'engrams', in mouse Dentate Gyrus (DG). The DG has been shown to encode engrams of emotionally valenced experiences and is necessary for spatial working memory tasks such as the Delayed-Non-Match-to-Place (DNMP) task. Male and female wild type mice were trained to perform the DNMP task on a T-maze for water reward. Using the fos tet-tag activity-dependent labeling system, DG engram ensembles were tagged with eGFP as mice explored a novel T-maze context. In one experiment, animals were re-exposed the following day to the same maze context, a subsection of that maze, or a novel open field context. Immunohistochemical staining for the colocalization of eGFP and c-Fos revealed the degree of overlap between the initial DG spatial engram and the ensemble representation during subsequent exposure to the different maze contexts. In a second experiment, DG granule cells active on a single route of the T-maze were tagged with the excitatory opsin and fluorescent label, ChR2-eGFP. Over 5 days of re-exposure to the full DNMP T-maze context, 20Hz pulsatile optogenetic stimulation was delivered bilaterally to DG during different sample phases of the task. Within-subjects analysis of trial accuracy, speed, and choice point occupancy were used to assess the behavioral outcomes of artificial spatial engram reactivation during ongoing navigation. These results extend previous findings on the role of the DG in spatial working memory tasks and the nature of episodic spatial experience used in subsequent neural representations and memory-guided decision making for navigation.

Disclosures: L.K. Wilmerding: None. **I. Kondratyev:** None. **S. Ramirez:** None. **M.E. Hasselmo:** None.

Poster

P866. Place Cells I

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Topic: H.09. Spatial Navigation

Support: CIHR Project Grant 367017
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Canada Research Chairs Program
NSERC Postgraduate Scholarship R256733C0G

Title: Decoding the neuronal basis of paired-associate learning strategies in mice

Authors: *Z. HAQQEE[1], E. WILSON[2], S. WILLIAMS[3], M. P. BRANDON[3];
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Abstract: Standardized behavioural tests offer a means to reliably translate experiments across laboratories, animal models, and even to clinical human populations. Touchscreen operant conditioning chambers are specifically designed with these advantages in mind. One behavioural test designed to isolate spatial memory processes uses an object location memory task in a touchscreen-automated operant conditioning chamber. This object-location paired-associate learning (PAL) task tests location-dependent associative memory performance in mice using touchscreen-automated operant conditioning chambers. The task is sensitive to hippocampal manipulations, with post-, but not pre-acquisition lesions to the hippocampus causing significant impairment in subsequent performance in mice. Here, we explore behavioural and neurophysiological changes as mice learn and perform the PAL task using miniaturized microscopes and one-photon calcium imaging of the hippocampus. We observe that mice developed increasingly stereotyped trajectories to solve the PAL task across time, which coincided with increasingly robust task-dependent calcium activity and spatial tuning in the hippocampus. In well trained mice, spatial tuning was conjunctive for trial-type-specific behavioral performance. Interestingly, task-tuned neural activity became more predictive of behaviour over multiple timescales, both within a recording session and across days. These data suggest that the hippocampus forms a gradual representation of object-location associations that reflect a stereotyped learning strategy.

Disclosures: Z. Haqqee: None. **E. Wilson:** None. **S. Williams:** None. **M.P. Brandon:** None.

Poster

P866. Place Cells I

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Program #/Poster #: P866.03

Topic: H.09. Spatial Navigation

Support: CIHR Project Grant #367017
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Canada Research Chairs Program

Title: Population dynamics of spatial reorientation in the thalamic head direction system

Authors: *Z. AJABI[1], A. T. KEINATH[1], X. WEI[3], M. P. BRANDON[2];
[2]Psychiatry, [1]McGill Univ., Montreal, QC, Canada; [3]Univ. of Texas at Austin, Austin, TX

Abstract: The mammalian head direction (HD) system is commonly referred to as a neural compass. However, contrary to a mechanical compass, the HD system does not have a universal across-environment reference frame. Instead, the internal HD representation is anchored to local environmental cues such that a rotation of these cues causes a similar rotation of the whole

network. Here, we study the population dynamics that underlie how the head direction system is anchored to visual sensory information. We recorded up to 239 HD neurons, simultaneously, using a head-mounted miniaturized microscope, in unrestrained mice. We designed experiments that alternated between light and dark conditions, while a single visual cue was shifted from one location to another. These experiments caused rapid rotations of the HD network (resets) in order to realign with the changing direction of the cue. We defined a 'network gain' that reflected the neural population activity and found that modulation of this quantity was highly correlated with reset behavior (i.e. speed and range). On the other hand, darkness periods were associated with slower, yet, predictable drifts of the internal HD representation which seemed to revert to its baseline configuration, even after cue manipulation. This phenomenon was accompanied by a remarkable change in the network gain landscape, showing peaks of activity at the internal location not only of the immediately preceding cue but also those further in the past. These patterns highly depended on the duration of cue exposure and so, may indicate the involvement of synaptic plasticity in maintaining neural traces of salient environmental cues. This internal alternation between the visual reference frame, in light conditions, and baseline reference frame, during darkness, was also confirmed with a continuously rotating visual cue. Notably, this manipulation caused persistent drift biases, in darkness, matching the direction and speed of cue rotation, which shows the important role of the optic flow in calibrating the integration of vestibular information in the head direction system.

Disclosures: Z. Ajabi: None. **A.T. Keinath:** None. **X. Wei:** None. **M.P. Brandon:** None.

Poster

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NIH R01 MH052090
ONR MURI N00014-19-1-2571

Title: Neural correlates of environmental alterations in retrosplenial cortex and high-resolution infrared tracking of behavior

Authors: *L. C. CARSTENSEN[1], A. S. ALEXANDER[1], G. W. CHAPMAN, IV[2], A. J. LEE[1], M. PATEL[1], Y. GU[1], M. JALAL[1], M. BETKE[1], M. E. HASSELMO[2];
[2]Psychological and Brain Sci., [1]Boston Univ., Boston, MA

Abstract: Flexible and efficient spatial navigation through the external world and the ability to adapt to changing environments is important for many complex behaviors. The retrosplenial cortex (RSC) is an association area that is interconnected with numerous regions of the brain important for spatial memory and navigation, including the hippocampal formation (HPC), rhinal cortices, and anterior thalamic nuclei. Neurons in these regions are known to display diverse spatial correlates, such as encoding the past or present location of objects, proximity to objects or boundaries, and position in the observable environment. Additionally, studies in both rodents and humans have shown that RSC is important for spatial memory, such as tracking distance from a location or landmark, contextual information about a known environment, orientation within an environment, and knowledge of distances between objects. These reasons and the connectivity of RSC show the importance of determining precisely how RSC neurons represent available cues such as objects or boundaries and their relationships within the local environment. In the current work, we performed in vivo electrophysiological recordings in RSC while rats free foraged in arenas that could contain an object or in which the local environment was altered. Building upon previous work, we report that RSC neurons display systematic changes in mean firing rate responding to alterations of the environment. These alterations include the boundaries of the arena rotating, changing size or shape, or an object being introduced into the arena. These results distinguish RSC as a region important in orientation and mapping features of spatial environments. Separate from our neural recordings in RSC, we analyzed high-resolution infrared behavioral tracking data of head and body orientation and position to better understand the relationship between elements of behavioral pose including head direction, body direction, movement direction and speed of the animal. This will allow for more in-depth analyses of the neural correlates of behavioral pose, movement, and directional relationships in future studies of animal behavior.

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Poster

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Support: NIH R01 MH120073
NIH R01 MH060013
NIH R01 MH052090
NIH F32 NS101836
NIH K99 NS119665

ONR MURI N00014-19-1-2571

Title: Laminar and projection-specific calcium imaging of spatial memory related retrosplenial cortex dynamics in freely moving rats

Authors: *A. S. ALEXANDER, B. B. SCOTT, M. E. HASSELMO;
Ctr. for Systems Neuroscience; Dept. of Psychological and Brain Sci., Boston Univ., Boston, MA

Abstract: Retrosplenial cortex (RSC) neurons encode a variety of spatial variables in allocentric, route-centric, and egocentric coordinate systems (Alexander et al., 2015; 2017; 2020). Lesions or inactivation of the area indicate that it is important for navigation and mnemonic processing. RSC activation supporting these processes in freely moving rats has primarily been characterized using in vivo electrophysiology. One-photon calcium imaging in behaving animals allows observation of larger populations of simultaneously recorded neurons. In retrosplenial cortex, imaging large populations affords an examination of interactions amongst neurons with activity anchored in distinct spatial coordinate systems which could reveal critical processing modes for the construction and use of internal maps for navigation and memory. In the current work, we utilized in vivo one-photon calcium imaging in Thy1-GCaMP6f transgenic rats to characterize population dynamics of hundreds of retrosplenial cortex neurons while animals learned and performed multiple tasks requiring spatial memory. We gained optical access to RSC ensembles by using microprism lenses which enabled a comparison of activation between deep and superficial layers of the region during behavior. Finally, retrograde-mediated transduction of GCaMP7 was used to investigate differences and similarities in task-related calcium signals between RSC neurons that project to the anterior thalamic nuclei or the extended hippocampal formation.

Disclosures: A.S. Alexander: None. **B.B. Scott:** None. **M.E. Hasselmo:** None.

Poster

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Program #/Poster #: P866.06

Topic: H.09. Spatial Navigation

Support: DFG Research Fellowship #OU 135/1-1
CIHR Project Grant #367017
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Canada Research Chairs Program

Title: Dissociating object versus spatial pattern separation: frombehavior to neuronal activation

Authors: *M. OULÉ[1,2], C. P. KRAMAR[1,2], A. T. KEINATH[1,2], L. M. SAKSIDA[3], T. J. BUSSEY[3], M. P. BRANDON[1,2];
[1]McGill Univ., Montreal, QC, Canada; [2]Psychiatry, Douglas Res. Ctr., Verdun, QC, Canada; [3]Western Univ., London, ON, Canada

Abstract: The large number of mature granule cells and the presence of neurogenesis in the dentate gyrus (DG) have led to the hypothesis that this region is especially sensitive to small changes in input, making it particularly important when encoding highly similar stimuli, a processed named 'pattern separation'. The entorhinal cortex (EC) is the main source of cortical inputs to the DG: while its medial segment (MEC) preferentially forwards spatial information, its lateral part (LEC) rather sends object-related information. The contribution of mature and immature granule cells to spatial versus object pattern separation remains less clear. While recent work has made important progress to understand the network-level computation that underlies spatial pattern separation in the DG, very little is known about the physiology and contribution of each type of granule cell to support object pattern separation. To address this question, we established a new task that specifically assesses object pattern separation and evaluate the role of immature granule cells in this task. Using c-fos immunofluorescence labeling of activated neurons, we studied the activation of different brain regions in the object pattern separation compared to well-established spatial pattern separation tasks. We further seek to use the UCLA miniscope to study – before and after ablation of immature granule cells – how the DG differentially processes object versus spatial pattern separation.

Disclosures: M. Oulé: None. **C.P. Kramar:** None. **A.T. Keinath:** None. **L.M. Saksida:** None. **T.J. Bussey:** None. **M.P. Brandon:** None.

Poster

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Topic: H.09. Spatial Navigation

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NIMH Grant: MH05209
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Title: The Hippocampal Representation of Space is Modulated by the Availability of Visual Information

Authors: *D. J. SHEEHAN[1], B. A. FORDYCE[2], A. S. ALEXANDER[3], M. E. HASSELMO[4]; [1]Boston Univ. CILSE, Boston, MA; [2]MGH, Boston, MA; [4]Psychological and Brain Sci., [3]Boston Univ., Boston, MA

Abstract: Abstract: In mammalian species the hippocampus and associated regions have been demonstrated to be critical and necessary for many forms of complex navigation, especially memory guided wayfinding. Hippocampal place cells are believed to be a component of the neural substrate onto which ongoing active experience can be mapped. While many path-integration based theories of navigation focus on the internalized drive in creation of place fields, manipulations regarding the external state of the environment have often resulted in alterations of place cell coding. Further, spatially receptive fields have been shown to be sensitive to various reference frames, which can be externally or internally anchored. In this study here we employ a linear track task with tetrode recording of the spiking activity of principal cells of the dorsal CA1 region of the rat hippocampus. Recordings were performed during outward and inward runs in epochs with or without visual information being present. We show here that the hippocampus is sensitive to environmental lighting manipulations that do not signal a change in task structure or behavioral demands, indicating that hippocampal representations of space are modulated by availability of visual stimuli and optic flow.

Disclosures: D.J. Sheehan: None. **B.A. Fordyce:** None. **A.S. Alexander:** None. **M.E. Hasselmo:** None.

Poster

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Topic: H.09. Spatial Navigation

Support: NIH R01 MH120073
MH060013
MH052090
N00014-19-1-2571
CIHR Project Grant #367017
CIHR Project Grant # 377074
NSERC Discovery Grant # 74105

Title: Examining the role of medial septal cell types in entorhinal grid cell generation

Authors: *J. C. ROBINSON[1], M. P. BRANDON[2], M. E. HASSELMO[1]; [1]Psychological and Brain Sci., Boston Univ., Boston, MA; [2]Psychiatry, McGill Univ., Montreal, QC, Canada

Abstract: The medial entorhinal cortex (MEC) is a cortical region critical for spatial navigation and memory processing. The MEC contains several cell types which together represent different spatial aspects of the current environment; these neurons include 1) grid cells which fire at distinct locations that form a repeating hexagonal pattern, 2) boundary vector cells which fire when close to the boundaries of the spatial field, and 3) head direction cells which fire in relation to the animal's head orientation. Input from the medial septum (MS) is essential for MEC function. Inactivation of the entire MS results in the complete abolition of grid cell spatial firing. The MS is made up of three distinct populations: cholinergic, GABAergic and glutamatergic neurons. Here we have evaluated the role of MS GABAergic and glutamatergic neurons in grid cell generation. We have specifically targeted either GABAergic or glutamatergic neurons in the MS using optogenetic activation of Archaerhodopsin to selectively silence each cell type while recording grid cells in the MEC. For both groups, an optic fiber was placed above the MS for light delivery, and a four-tetrode microdrive was implanted into the MEC for grid cell recordings. Our results show that silencing septal glutamatergic neurons did not affect MEC theta power nor frequency; however, silencing of this population resulted in a distortion and an overall broadening of individual grid fields. Next, we found that optogenetic silencing of septal GABAergic neurons resulted in up to a 60% reduction in MEC theta power. We used two stimulation protocols: 30 second inactivation followed by a 30 second recovery period as well as a 30 second inactivation period followed by a 60 second recovery period. Grid cell spatial firing was disrupted during both the 30 second laser on and 30 second laser off period. However, with a longer recovery period between inactivations (60 seconds), grid cell spatial firing was mostly maintained with only subtle spatial distortions to the grid pattern. Preliminary results suggest that the extent of the distortion to the grid cells may be related to degree of spatial periodicity during baseline recordings. Cells with a higher gridness score may be more resilient to septal GABAergic inactivation. These results reveal an important role of MS GABAergic neurons in grid cell spatial firing, while MS glutamatergic neurons appear to play a more subtle role in modulating the grid cell spatial pattern.

Disclosures: J.C. Robinson: None. **M.P. Brandon:** None. **M.E. Hasselmo:** None.

Poster

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Title: The long-term dynamics of the CA1 spatial code preserve the relative structure of contextual representation

Authors: *A. T. KEINATH, C.-A. MOSSER, M. P. BRANDON;
McGill Univ., Verdun, QC, Canada

Abstract: The hippocampal formation is thought to mediate episodic memory through the instantiation and reinstatement of context-specific cognitive maps. However, recent longitudinal experiments have challenged this view. These studies find that the majority of hippocampal cells change their tuning properties over a timescale of days even in the same environment, yielding maps of the same context that are as different across time as maps of different context when assessed by various population-level measures. Despite these population-level dynamics, a minority of cells can maintain their tuning properties across a months-long timescale, at least under some circumstances. Often referred to as *drift*, these dynamics raise a variety of questions about the representational capacity and content of the hippocampal code. One critical question is whether and how these dynamics impact the contextual code. On one hand, such dynamics might lead to a reorganization of the contextual representation, whereby the representation of each context drifts independently of other contextual representations. On the other hand, if drift is coordinated across contexts then such dynamics might instead preserve the relative structure of contextual representation, allowing consistent readout of contextual information across long timescales. To adjudicate between these possibilities, we imaged large CA1 populations over more than a month of daily experience as freely behaving mice explored six differently-shaped environments in an extended geometric morph paradigm. This paradigm elicited partially-correlated population-level maps, allowing us to characterize the evolution of the relative representational structure over long timescales. We found that individual cells represent context to varying degrees through heterogenous but stereotyped changes to their spatial tuning properties, leading to an attractor-like population response which develops over the course of extended experience. Characterizing the full representational structure revealed that long-timescale changes in population activity occurred orthogonally to the representation of context in network space. Thus, despite continued representational changes over the course of the experiment, the relative representational structure of context was preserved, allowing for consistent readout of contextual information across weeks. Together, these results demonstrate that long-timescale changes to the CA1 spatial code preserve the relative structure of contextual representation, a result which holds multiple fundamental theoretical implications.

Disclosures: A.T. Keinath: None. **C. Mosser:** None. **M.P. Brandon:** None.

Poster

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Topic: H.09. Spatial Navigation

Support: CIHR Project Grant #367017
CIHR Project Grant # 377074
NSERC Discovery Grant # 74105
Canada Research Chairs Program

Title: Spatial coding of topologically deformed environments in CA1 across protracted experience

Authors: *J. Q. LEE[1], A. T. KEINATH[1], E. CIANFARANO[2], M. P. BRANDON[2];
[2]Psychiatry, [1]McGill Univ., Montreal, QC, Canada

Abstract: Recent modelling and empirical studies present multiple alternative views on the spatial encoding of environments in the hippocampus. While classic models on hippocampal mapping suggest a euclidean spatial metric determines place cell firing, others suggest a non-euclidean embedding, or that the hippocampus encodes predictive topological relationships. Adjudicating between these models requires a rich dataset capable of quantitatively assessing the unique and often nuanced predictions distinguishing each model. To this end, we performed a series of systematic topological deformations of a familiar space over a month-long period in freely-behaving animals while recording large neuronal populations in mouse CA1 with miniscope calcium imaging. Next, we leverage these data to quantitatively assess euclidean, non-euclidean, and predictive models of hippocampal mapping via representational similarity analysis (RSA) - an approach that allows for systematic characterization and comparison of data and models alike. Our results characterize the hippocampal representation of environmental connectivity with a new level of precision, and will serve as a benchmark by which to judge existing and future models of hippocampal mapping.

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Poster

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Program #/Poster #: P866.11

Topic: H.09. Spatial Navigation

Support: CIHR Project Grant #367017

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Canada Research Chairs Program

Title: Hippocampal neurons encode behavioral space

Authors: *M. YAGHOUBI[1,2], C. MOSSER[2], A. NIETO[2], T. GISIGER[2], E. WILSON[2], S. WILLIAMS[2], M. P. BRANDON[2];
[1]Integrated Program in Neurosci. (IPN), McGill Univ., Montreal, QC, Canada; [2]Dept. of Psychiatry, McGill University, Douglas Hosp. Res. Ctr., Montreal, QC, Canada

Abstract: The cognitive map theory of hippocampal function has largely focused on mapping the allocentric spatial dimensions of the external world [1]. More recent evidence points to a broader role of the hippocampus in forming maps for other non-spatial aspects of the environment [2,3]. Beyond encoding allocentric space or external salient features, we ask if the hippocampus encodes an animal's behavioral repertoire after they become an expert in solving a difficult-to-learn delayed non-match to location task. We specifically explore whether the hippocampus preferentially encodes behavioral sequences used to solve the task (behavioral space) over allocentric space. We recorded the activity of up to one thousand individual hippocampal neurons as well-trained mice perform the Trial-Unique Nonmatch to Location (TUNL) touchscreen task [4]. Here, a white square is presented in one of five positions on the touchscreen. A nose poke to this square makes it disappear and starts a delay period (increasing from 2 to 8s as the mice learn the task). Following the delay, two white squares are displayed, and the mouse must choose the nonmatching square to receive reward located on the back wall of the touchscreen chamber. In contrast to prior work that describes behavior with a few variables such as position, reaction time, etc, here we develop and apply a deep-learning-based approach that clusters and quantifies the transition statistics between sequences of egocentric body movements. This pipeline is subtle enough to reveal individual differences between mice. CA1 neurons were spatial tuned yet, our analysis show that egocentric behavioral information is strongly encoded and is better correlated with task performance than allocentric spatial information. Decoding analysis revealed that behavioral choices can be predicted from either neuronal activity or behavior many seconds before decisions are executed. While egocentric behavioral sequences could predict future choice, decoding of future choice from neural activity outperformed behavioral decoding during the sample, delay, and choice phases of the TUNL task, providing evidence for a prospective neural memory trace that is independent of mouse behavior. Together, our results suggest that, in well-trained animals, the hippocampus encodes behavioral space more reliably than allocentric space, which may function to support memory-guided behavior to solve the TUNL task. [1] O'Keefe & Dostrovsky, 1971. [2] Aronov et al., 2017. [3] Pastalkova et al., 2008. [4] Kim et al., 2015.

Disclosures: M. Yaghoubi: None. **C. Mosser:** None. **A. Nieto:** None. **T. Gisiger:** None. **E. Wilson:** None. **S. Williams:** None. **M.P. Brandon:** None.

Poster

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Title: Investigating emergent hippocampal dynamics through standardized high-throughput behavior and neuronal imaging using the McGill-Mouse-Miniscope platform

Authors: *C.-A. MOSSER, M. YAGHOUBI, A. NIETO-POSADAS, Z. HAQQEE, T. GISIGER, E. WILSON, S. WILLIAMS, M. P. BRANDON;
Psychiatry, McGill University, Douglas Hosp. Res. Ctr., Montréal, QC, Canada

Abstract: Recent technical advances enable the recording of large neuronal populations during behavior resulting in increasingly complex datasets. Despite bold and important open-science and data-sharing policies, these datasets across laboratories tend to apply unique data acquisition methods, behavior, and file structures. Discrepancies between protocols present key challenges including the comparison of results between brain regions, laboratories, and species. We have established the McGill-Mouse-Miniscope platform (M3, www.m3platform.org) to combine miniscope calcium imaging with standardized touchscreen-based behavioral testing. Our mission is to curate an open-source and standardized framework for acquiring, analyzing, and accessing high-quality data of the neuronal dynamics that underly behavior and cognition throughout the brain in mice, and in a near future marmosets. Each experiment provides up to 1000 simultaneously recorded neurons from a single brain region over the course of ~3 months as animals initially learn and master the task. We have collected several datasets to highlight the feasibility of this approach, including hippocampal CA1 and CA3 recordings during the delayed trial-unique nonmatching-to-location (TUNL) task. In this task, a white square is presented in one of five positions on the touchscreen. A nose poke to this square starts a delay period. Following this delay, two white squares are displayed, and the mouse must choose the nonmatching square to receive reward. As expected, CA1 and CA3 neurons are spatially modulated, however, they are also sensitive to other behavioral and task-related features. We found that 1) ~10% of neurons participate in sequences tiling the delay period, 2) delay sequences are unique to the sample square location, 3) the fidelity of sequence predict performance, and 4) delay sequence cells were reliably activated during reward. We are now examining how this structure emerges in CA1 and CA3 regions during the initial learning of this task, and how network activity restructures when memory load is changed. These data will help to shed light on how large populations of hippocampal neurons encode a complex memory task, and will allow for comparison across laboratories, mouse models, and species.

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Poster

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Program #/Poster #: P866.13

Topic: H.09. Spatial Navigation

Support: CIHR Project Grant #367017
CIHR Project Grant # 377074
NSERC Discovery Grant # 74105
Canada Research Chairs Program

Title: Optogenetic inactivation of septal GABAergic neurons causes remapping in time cells with no deficits in behavior.

Authors: *H. YONG[1], H. CHANG[3], M. P. BRANDON[2];
[1]Integrated Program in Neurosci., McGill Univ., Verdun, QC, Canada; [2]Psychiatry, McGill Univ., Montreal, QC, Canada; [3]Dept. of Neurosci., Univ. of Lethbridge, Lethbridge, AB, Canada

Abstract: Neurons in the hippocampus are sequentially activated during the delay period of a delayed spatial alternation task. This sequential readout is dependent on an intact medial septum and is hypothesized to require theta oscillations and to support behavioral performance. However, these conclusions are based on work that used a long-acting GABA receptor agonist to inhibit the activity of all medial septum neurons for the entire testing session, raising the possibilities that 1) septal contributions other than theta generation may support delay encoding, and 2) that memory deficits could be the result of septal inactivation outside of the delay period. To confront these possibilities, we applied an optogenetic strategy to selectively silence the theta-generating GABAergic population in the medial septum (MS) specifically during the delay period as mice performed the delayed spatial alternation task. Mice were trained to run on a treadmill for 10 seconds between each alternation lap on the T-maze. Septal GABAergic neurons were pseudo-randomly inhibited in 50% of the trials, for the entire 10 second duration of the delay period. We show that inhibition of these neurons significantly reduced the amplitude of hippocampal theta oscillation (mean theta reduction: 60%), and induced remapping in many of hippocampal neurons that encoded the delay period. Moreover, the decoding accuracy for trial identity significantly decreased with the inactivation. Thus, we observed a new delay sequence only on trials when the septal GABAergic input to the hippocampus was suppressed. These phenomena were not observed in laser-on GFP-only expressing control experiments. This result suggests the possibility that region CA1 of the hippocampus receives competing delay sequences from CA3 and the medial entorhinal cortex (MEC), both of which are known to contain delay

cells. We suggest the possibility that MS inactivation disrupts the MEC delay sequence (similar to the effect of MS inactivation on MEC grid cells), which then gives the CA3 input more influence over CA1 sequences. To our surprise, remapping of the delay sequence had no impact on behavioral performance on the T-maze delayed alternation task. Inhibition of MS GABAergic neurons outside of the delay period did not induce memory deficits either. This suggest several possibilities including 1) the identity of the sequence is not critical for performance, and/or 2) choice information is still preserved in the remapped sequence. We are exploring these possibilities.

Disclosures: H. Yong: None. **M.P. Brandon:** None.

Poster

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Topic: H.08. Learning and Memory

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Title: Three activity states of hippocampal principal neurons: the silent, primed and engaged

Authors: *X. CHEN, Y. ZHOU;
Univ. of New Hampshire, Durham, NH

Abstract: Memory-eligible neurons (or primed neurons) are a subset of sparsely distributed neurons in memory-related regions that can sustain long-term synaptic modifications to hold memories. Memory-eligible neurons “activation” during memory acquisition and “re-activation” during memory retrieval are widely thought to mediate memory formation and retrieval, respectively. Hence, memory-eligible neurons, like neurons mediating innate physiological function, are widely perceived to have two activity states: the silent and active. Here we show that hippocampal principal neurons exhibit at least three distinct activity states during trace fear conditioning. They are termed the silent, primed, and engaged states, as distinguished by their basal activity levels, activity plasticity, calcium entry, burst synchronization, and importantly, memory-engaging status. During the period when a small portion of primed neurons fulfill learning and mnemonic duties by transitioning back and forth between the primed and engaged states, most hippocampal CA1 principal neurons have very weak activity and stay in the silent state. Memory-eligible neurons in the primed state are sensitized with high excitability and exhibit irregular activity prior to training, in which they are primed to modify activity pattern

(and likely synaptic efficacy). Memory-eligible neurons in the engaged state are synergistically committed to encoding memory information and display burst synchronization and robust calcium entry. We have developed a computational method based on *in vivo* calcium imaging data in freely behaving mice to numerically define primed neurons. In the healthy brain, memory-eligible neurons can readily transition from the primed to the engaged state, and vice versa. Nevertheless, the transition kinetics between the silent and the primed states are much slower, likely because the primed-silent hierarchy among hippocampal neurons is well-maintained within a certain period. We propose that hippocampal memories are formed, not simply due to memory-eligible neurons "activation" directly from the silent state, rather because primed neurons with random dynamics are induced to form burst synchronization. That is a transition process from the primed to engaged state. Likewise, hippocampal memories are retrieved, not because of memory-eligible neurons "re-activation" from the silent state, rather because the same subset of primed neurons successfully transitions to the engaged state.

Disclosures: X. Chen: None. **Y. Zhou:** None.

Poster

P894. Learning and Memory in Aging: Animal Models II

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Topic: H.12. Aging and Development

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CIHR SIB78537
AHFMR 20060436

Title: Investigation of the effect of age and remapping status on the number of cell assemblies in hippocampus CA1 pyramidal cells

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Abstract: Normally aged rats express a reduced capacity to consolidate and recall episodic memories. Furthermore, hippocampal place fields in aged rats can undergo "spontaneous remapping," a phenomenon in which spatial representations reconfigure when animals are re-

exposed to a familiar environment. The neuronal mechanisms underlying age-associated deficits in memory consolidation and increased spontaneous remapping are not well understood. Cell assemblies, defined as anatomically and/or functionally connected groups of neurons that exhibit a temporal pattern of activity, are thought to represent mental and perceptual entities. We therefore hypothesize that age-associated memory decline and spontaneous remapping in aged rats are related to a reduction in the number of cell assemblies. We tested the hypothesis on single-unit recordings that were acquired from CA1 pyramidal cells in five young and six old rats while they learned a place-dependent eyeblink conditioning task (Schimanski et al., 2013). The number of cell assemblies was estimated by a recently proposed method (Russo and Durstewitz, 2017), which allows systematic detection of cell assemblies across a range of temporal bin sizes (3 ms to 100 ms). A preliminary analysis indicated that there may be more cell assemblies in young versus old rats and in non-remapping versus remapping rats; however, these results were obtained without separating behavior epochs from sleep epochs and remain to be validated. It also appeared that cell assemblies were most abundant during the first few days of eyeblink conditioning training, suggesting that we may find an effect of learning on the number of identified assemblies.

Schimanski, L. A., Lipa, P., & Barnes, C. A. (2013). Tracking the course of hippocampal representations during learning: when is the map required?. Journal of Neuroscience, 33(7), 3094-3106.

Russo, E., & Durstewitz, D. (2017). Cell assemblies at multiple time scales with arbitrary lag constellations. Elife, 6, e19428.

Disclosures: S.Y. Campbell: None. **J. Eseonu:** None. **K. Ali:** None. **L.A. Schimanski:** None. **C.A. Barnes:** None. **S.L. Cowen:** None. **M. Tatsuno:** None.

Poster

P894. Learning and Memory in Aging: Animal Models II

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Program #/Poster #: P894.02

Topic: H.12. Aging and Development

Support: McKnight Brain Research Foundation
R24 AG061421
R01 AG049465
S10 OD025016

Title: A test of the hypothesis that factors acting to protect synapse function are at the core of the biological basis of cognitive reserve

Authors: *D. T. GRAY[1], L. DO[2], S. O. KHATTAB[1], I. SINAKEVITCH[1], T. P. TROUARD[2], C. A. BARNES[1,3];

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Abstract: One hallmark of normative brain aging is the vast heterogeneity in whether older people succumb to or are able to resist age-associated cognitive decline. Such variability among people may arise in part as a result of some brain property that enables neuronal networks to maintain cognitive performance in the face of accumulated insults that could compromise brain function. This is known as cognitive reserve. For a brain property to be considered cognitive reserve, it must moderate the relationship between brain functions that change with advanced age and cognition. We assess the degree to which synapse health minimizes the cognitive burden of age-associated structural changes in grey and white matter utilizing legacy brain tissue from a large experiment designed to study the molecular mechanisms of cognitive aptitude in male F344 rats across the lifespan, including young (6-8 mo), middle-aged (15-17 mo), and old (23-25 mo) rats. All rats underwent a series of structural and diffusion-weighted MRI scans. Rats were categorized as average-, low-, or high-performing for their age group through a large battery of cognitive assessments of both medial temporal lobe and frontal cortical brain function. Histological hippocampal sections were prepared and labelled to identify two distinct proteins critical for synapse function: immediate-early gene neuronal pentraxin 2 (NPTX2) and markers for a specialized extracellular matrix structure called perineuronal nets (PNNs). In the MRI data we have uncovered several brain measures that change with advanced age but are not associated with the cognitive status of the rats: diffusion weighted MR analysis of fractional anisotropy, apparent diffusion coefficient, axial diffusivity, and radial diffusivity as scalar parameters. Whole brain and white-matter masked estimates of all these scalar parameters were different in the aged rats compared to the middle-aged and young rats, yet none showed an association with the cognitive status of rats within an age group. We present the results of a multiple regression model designed to assess the degree to which the expression of markers of synapse function moderates the relationship between these structural brain measures in the MRI and cognition. If synapse health is central to cognitive reserve, the expression of proteins associated with synapse function will be highest in the high cognitive functioning rats in all 3 age groups. This may indicate that synapse integrity at all points of the lifespan helps define the trajectory that an individual's cognitive function will take as age-associated changes in brain structure and function begin to accumulate.

Disclosures: D.T. Gray: None. **L. Do:** None. **S.O. Khattab:** None. **I. Sinakevitch:** None. **T.P. Trouard:** None. **C.A. Barnes:** None.

Poster

P894. Learning and Memory in Aging: Animal Models II

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Program #/Poster #: P894.03

Topic: H.12. Aging and Development

Support: McKnight Brain Research Foundatio
RO1 AG003376

Title: Aged rats display more accurate allothetic cue-based goal navigation in response to sudden changes in environmental cues

Authors: *A. W. LESTER[1], G. A. JORDAN[1], Z. P. PHILPOT[1], C. A. BARNES[1,2];
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Abstract: As with older adults, aged rats show pronounced impairments on a number of different spatial navigation tasks as well as a bias toward relying on self-motion (i.e., idiothetic) over environmental (i.e., allothetic) cue-based navigation strategies (Lester et al., 2017). The Instantaneous Cue Rotation (ICR) task used here requires animals to navigate to a reward location that is always aligned to the projected visual cues in the environment (Lester et al., 2018). By instantaneously rotating the cues we are able to put allothetic and idiothetic feedback in direct and immediate conflict and characterize how quickly and accurately young and aged animals utilize environmental cues to navigate to a new rotated goal location. Behavioral data from 10 young and 10 aged rats suggest that both old and young rats were able to utilize allothetic cues to navigate to a cue aligned goal, even when cues were suddenly rotated during ongoing behavior. Results from the young rat cohort indicated that, while the behavior of these animals was reliably influenced by allothetic cue rotation, they also showed a tendency to overshoot the goal, even during baseline trials (i.e., before cues were rotated). Additionally, following cue rotation, young rats tended to slightly under-rotate relative to the cues. That is, when cues were rotated backward or forward, young rats tended to stop at a position that was just past or ahead, respectively, of where they tended to stop prior to the cues being rotated. The behavior of the aged rats, in contrast, was more accurate on average and showed more complete control by the cues. Comparisons of the velocity profiles during periods surrounding cue rotation from the two age groups indicate that aged rats tended to show a greater decrease in running speed immediately following cue rotation and that the degree to which animals from either age cohort decelerated following cue rotation was predictive of their subsequent goal navigation accuracy. Taken together, these findings suggest that aged rats may actually demonstrate more accurate allothetic cue-based goal navigation, possibly because they are more attentive to changes in the environmental cues.

Disclosures: A.W. Lester: None. **G.A. Jordan:** None. **Z.P. Philpot:** None. **C.A. Barnes:** None.

Poster

P894. Learning and Memory in Aging: Animal Models II

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Program #/Poster #: P894.04

Topic: H.12. Aging and Development

Support: McKnight Brain Research Foundation
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CIHR SIB78537
AHFMR 20060436

Title: Aged rats exhibit a reduced hippocampal gamma frequency response to changes in running speed, altered spatial information in place fields, and increased spike-field coherence

Authors: *L. M. CROWN[1], D. T. GRAY[1], L. A. SCHIMANSKI[4,1], C. A. BARNES[1,2], S. L. COWEN[3,1];
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Abstract: Hippocampal gamma (30-120 Hz) and theta (5-10 Hz) oscillations are associated with mnemonic and navigational processes. An important property of these oscillations is that they adapt to changes in an animal's behavioral state to optimize the processing of spatial information. For example, it has been shown that gamma and theta frequencies increase with increasing running speed. Whether the dynamic scaling of gamma frequencies with behavior is altered with age has not yet been investigated. In this study, we examined local-field and place cell activity in the hippocampus CA1 region of five old and six young rats as they performed a spatial eye-blink conditioning task across 31 days. We found that while mean gamma frequency was not different between age groups, the slope of the increase in gamma frequency with increasing running speed was shallower in old rats. By contrast, theta frequencies scaled with running speed similarly in young and aged rats, although overall theta was shifted to lower frequencies in aged rats. We also found that place cells in aged animals have reduced spatial information content per field accompanied by reduced within-field, but not out-of-field, firing rates. Controlling for reduced firing rates, we found that spatial information per spike was significantly greater in older rats. Finally, we observed that spike phase-locking to gamma, but not theta, was greater in older animals. Together, these results highlight the importance of accounting for locomotor activity and oscillatory dynamics when analyzing electrophysiological data as well as indicate a dissociable impact of age on theta- and gamma-generating mechanisms. Changes in the responsiveness of gamma and theta oscillations to locomotor activity could contribute to altered navigational performance in older individuals.

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Poster

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Program #/Poster #: P894.05

Topic: H.12. Aging and Development

Support: John G. Kulhavi Professorship in Neuroscience at CMU
Field Neurosciences Institute

Title: Progesterone-induced reductions of age-related deficits in cognition and long-term potentiation in C57BL/6 male mice.

Authors: *S. BANERJEE[1], K. JENROW[1], J. ROSSIGNOL[2], R. BOSE[1,3], G. L. DUNBAR[1,4]; [1]Psychology, Neurosci. Program, Field Neurosciences Inst. Lab. for Restorative Neurol., [2]Neurosci. Program, Col. of Medicine, FNI Lab. for Restorative Neurol., Central Michigan Univ., Mount Pleasant, MI; [3]Neurosci., Karolinska Inst., Stockholm, Sweden; [4]Field Neurosciences Inst., Ascension St. Mary's Hosp., Saginaw, MI

Abstract: Progesterone functions as both a sex hormone and a neurosteroid, with proven neuroprotective effects in both male and female animals. Because serum progesterone levels decline with age in human males, we hypothesized that the exogenous administration of progesterone would reduce age-related deficits in cognition in male mice. To test this, we used groups of young (4-month-old) and aged (24-26-month-old) male C57BL/6 mice. Daily subcutaneous injections of progesterone (5 mg/kg) or equivalent volumes (200µl) of vehicle (30% 2-hydroxy beta-cyclodextrine) were administered to mice for 21 consecutive days. Cognitive functioning was assessed using a water-T-maze reversal task during the entire 21-day treatment period. Briefly, mice had to locate a hidden platform in one of two arms of the T-maze, in which the location was switched once the mouse achieved an 80% success rate in a single day. At the end of the 21-day treatment period, long-term potentiation (LTP) was assayed *in vivo* in the dentate gyrus (DG) following theta-burst stimulation of the medial perforant path. After completing the LTP assay, mice were euthanized to collect and measure serum progesterone levels and LTP-induced immediate early gene (IEG) expression in the ipsilateral and contralateral hemispheres. Results revealed that aged, vehicle-treated mice attained fewer reversals and required more days to complete each reversal than young vehicle-treated mice. LTP was also impaired in these aged mice. Progesterone improved the performance of aged mice, resulting in an increase in number of reversals attained and requiring fewer days to complete each reversal. LTP was also improved and was closer to that observed in young vehicle-treated mice. No significant changes were observed in young mice receiving progesterone treatments. Measures of serum progesterone levels and IEG expressions are ongoing and are hypothesized to correlate with the behavioral and LTP data. Our findings suggest that progesterone may be a potential therapy for age-related cognitive deficits.

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Poster

P894. Learning and Memory in Aging: Animal Models II

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Program #/Poster #: P894.06

Topic: H.12. Aging and Development

Support: McKnight Brain Research Foundation
RO1 AG049464
RO1 AG049465
S10 OD025016

Title: Global and regional and diffusion-weighted MRI analysis of rodent brains as a function of age and cognition

Authors: *L. DO[1], M. ZEMPARE[2], A. S. BERNSTEIN[1], P. K. BHARADWAJ[3], N. J. CAREY[2], C. NGUYEN[2], G. E. ALEXANDER[3,2], C. A. BARNES[2,4], T. P. TROUARD[1,2]; [1]Biomed. Engin., [2]Evelyn F. McKnight Brain Inst., [3]Dept. of Psychology, [4]Departments of Psychology, Neurol. and Neurosci., Univ. of Arizona, Tucson, AZ

Abstract: Translational studies of rodent brains have been and will continue to be crucial to characterizing the aging human brain. Magnetic Resonance Imaging (MRI) can quantitatively measure anatomy, connectivity and microstructure in both human and rodent brains and serves an important role in comparative studies of humans and rodents. Diffusion-weighted MRI (DMRI), in particular, is an established tool for the examination of white matter microstructure and connectivity and has been used to understand the effects of age and brain pathology. In the current work, adult male F344 rats were studied at three different ages: young (6mo), middle-aged (15mo) and aged (23mo). Cognitive performance of each rat was measured using a battery of behavioral tasks over the course of six weeks, including the Morris watermaze to test spatial and working memory, and the spontaneous object recognition task and a spatial location novelty and temporal ordering task. The spatial watermaze was used to categorize each animal as high, average or low cognition for its age group. DMRI of each rat was carried out at 7T and scalar metrics, including fractional anisotropy (FA), apparent diffusion coefficient (ADC), radial diffusivity (RD), and axial diffusivity (AD), were calculated. Additionally, fiber density and cross section was calculated using constrained spherical deconvolution and fixel-based analysis. ANOVA test of global DTI measures revealed a significant effect of age-group with follow-up pairwise comparisons revealing a small but significant decrease in FA, ADC, RD and AD between young and aged and middle-aged and aged, but no significant difference between young and middle-aged animals. There was no significant difference in global FA, ADC, RD and AD between any cognitive groups. Regional and voxel-based analysis is currently being carried out

and will characterize age-related changes in important learning centers of the brain and their relationship to cognitive performance.

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Poster

P894. Learning and Memory in Aging: Animal Models II

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Program #/Poster #: P894.07

Topic: H.12. Aging and Development

Support: McKnight Brain Research Foundation
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P51 RR000169

Title: Higher microglia densities are associated with reduced perineuronal net accumulation around parvalbumin-expressing neurons in the retrosplenial cortex of memory-impaired aged macaques

Authors: *S. KHATTAB[1], D. T. GRAY[1], K. E. MCDERMOTT[1], I. SINAKEVITCH[1], R. SCHWYHART[1], W. HARTIG[3], C. A. BARNES[1,2];
[1]Evelyn F. McKnight Brain Inst., [2]Departments of Psychology, Neurology, and Neurosci., Univ. of Arizona, Tucson, AZ; [3]Univ. Leipzig, PFI, Leipzig, Germany

Abstract: Altered synaptic plasticity is thought to play a central role in age-associated cognitive decline. Microglia have been shown to regulate synaptic plasticity through a number of mechanisms including remodeling and engulfment of extracellular matrix (ECM) structures (Crapser et al., 2019; Nguyen et al., 2020). With advanced age microglia show a number of phenotypic changes suggesting that they become more active compared to microglia in younger animals (Conde & Streit, 2006; Spittau, 2017). One such change is an increase in density indicating a greater proliferation. Perineuronal nets (PNNs) are specialized ECM structures that preferentially surround parvalbumin (PV) neurons, which are thought to regulate aspects of synaptic plasticity (Sorg et al., 2016). In rodents, there is limited evidence that fewer neurons are associated with PNN structures in aged animals compared to young. We sought to investigate whether 1) similar reductions occur in the brains of aged primates, and 2) whether changes in PNNs accumulation are associated with an animal's cognitive abilities. Our goals were to further understand the contributions of PNNs and microglia densities to age-associated cognitive decline and determine whether there is a relationship between microglia densities and PNN accumulation in older brains. To these ends, we used fluorescence labeling and unbiased quantification of the

PNN markers Wisteria floribunda agglutinin (WFA) and Aggrecan (ACAN), as well as the microglial marker IBA1 on the brains from a colony of 30 rhesus macaque monkeys ranging in age from 7 to 32 years (human equivalent ~21 - 96 years). All monkeys underwent tests of spatial short-term memory, object recognition memory, and object discrimination, which allowed analyses of relationships between the density of PNNs, microglia expression and cognition. Aged animals had a lower proportion of PV cells associated with WFA+ and ACAN+ PNNs in the retrosplenial cortex (RSC) than did younger animals, although total WFA and ACAN accumulation was not different. This decrease was also correlated with impaired performance specifically on object-based memory tasks but not on spatial memory tasks. Microglia densities were higher in aged animals and were associated with poorer performance on the object-based memory tasks. Importantly, a negative relationship emerged between microglia density and the proportion of PV-containing cells associated with ACAN. These results suggest that enhanced microglial activity with age may contribute to an age-associated degradation in ECM structures that has a circuit-specific impact on cognition in aged monkeys, likely through a destabilization of synapses.

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Poster

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Program #/Poster #: P894.08

Topic: H.12. Aging and Development

Support: McKnight Brain Research Foundation
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P51 RR000169

Title: Locus coeruleus neuronal, glial, and vascular populations remain stable with age in cognitively impaired rhesus macaques

Authors: *K. MCDERMOTT[1], I. SINAKEVITCH[1], D. T. GRAY[1], S. O. KHATTAB[1], W. P. PYON[4], W. I. SCHNAPP[2], C. A. BARNES[1,3];
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Abstract: The Locus Coeruleus (LC) is a brainstem nucleus best known for being the primary central nervous system site of noradrenaline production and is involved in the modulation and optimization of numerous behaviors in mammals. The LC is a molecularly and anatomically

heterogeneous region that is densely innervated by blood vessels and glial cells. The LC appears to be especially vulnerable to age-related neurodegeneration and is one of the first regions to show evidence of pathological changes associated with Alzheimer's disease (Mather & Harley, 2016). It is unclear, however, whether neurodegeneration of the LC is specific to neurodegenerative diseases such as Alzheimer's, or if LC neuron loss is part of the normal aging process. Our research utilizes 30 μm coronal brainstem sections from a colony of 30 cognitively assessed rhesus macaques ranging in age from 7 to 32 years (human equivalent ~21 - 96 years). All monkeys underwent tests of spatial short-term memory (delayed response), object recognition memory (delayed nonmatching-to-sample), and object discrimination. We used immunofluorescence techniques to identify neuronal nuclei (NeuN), catecholaminergic neurons (TH), vasculature (STL), and astrocytes (GFAP). The entirety of the locus coeruleus region for each immunolabeled section was imaged bilaterally at 40X on a high-resolution confocal microscope to obtain 3-dimensional volumes of the entire rostrocaudal extent of the LC. Neuron, glial cell, and vascular densities were determined using unbiased stereological techniques. These data were then assessed with respect to the age and cognitive status of the monkeys. Preliminary results from partial LC populations from 30 animals indicate that overall TH+ density and total neuron density remain stable in the older monkeys compared to the adults. Furthermore, vascular and GFAP staining density were not different between the adult and aged animals. These data appear to indicate that cell loss, changes in vascular innervation, and increases in astrocyte reactivity do not occur in the LC region of aged macaques, and lend support to the hypothesis that LC degradation is a distinguishing trait between normative and pathological brain aging.

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Poster

P894. Learning and Memory in Aging: Animal Models II

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Program #/Poster #: P894.09

Topic: H.12. Aging and Development

Support: McKnight Brain Research Foundation
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Title: Age-related compartment-specific volume reductions in macaque locus coeruleus: a combined MRI, Nissl and Immunofluorescence study

Authors: *I. SINAKEVITCH[1], K. E. MCDERMOTT[1], S. O. KHATTAB[1], G. A. JORDAN[1], D. T. GRAY[1], C. A. BARNES[1,2];
[1]Evelyn F. McKnight Brain Inst., [2]Departments of Psychology, Neurol. and Neurosci., Univ. of Arizona, Tucson, AZ

Abstract: The Locus Coeruleus (LC) is a brainstem nucleus with the largest group of noradrenaline-producing neurons in the brain. Dysregulation of LC systems contributes to cognitive dysfunction in both healthy aged brains and brains that succumb to Alzheimer's disease. Notably, the LC is heterogeneous along both the rostral-caudal and dorsal-ventral axes with respect to neuron morphology, projection targets, and vulnerability to the impacts of normative brain aging and neurodegenerative disease. In particular, the rostral LC projects to cortical and hippocampal brain regions critical to cognition. In this study, we describe in detail the standardized 3D anatomy of the LC nucleus using Nissl stains, TH-immunoreactivity, and MRI data. To this end, 30 μm coronal brainstem sections were examined along the rostral-caudal axis of the LC from a colony of 30 cognitively assessed rhesus macaques ranging in age from 7 to 32 years (human equivalent ~21 - 96 years). Prior to perfusion, these animals underwent structural MRI scans. AMIRA software was employed to reconstruct the LC from the TH-immunofluorescence and Nissl stains and aligned with MRI data that was collected prior to the histological procedures. After careful analyses of the high-resolution images collected from Nissl-stained serial sections and sections immunoassayed with anti-TH antibodies, we identified three distinct subnuclei in the macaque LC: a medial nucleus that is confined to the central gray area, a lateral nucleus that lays outside of the gray area laterally and abuts the mesencephalic tract (me5), and a central region within the LC medial nucleus that is characterized by densely packed TH-expressing neuronal somas. The volumes of these three LC compartments were delineated using TH+ cell bodies and large cell bodies in Nissl stains that are characteristic of this nucleus. Preliminary data from 23 animals indicate that the macaque LC extends approximately 2000 μm along the rostro-caudal axis. We observed a core LC nucleus with high TH+ cell densities that became more scattered in more rostral brain sections. Overall volumes that comprised both lateral and medial LC nucleus varied between 1.5 and 3 mm^3. Older monkeys tended to have smaller LC medial nucleus volumes than younger individuals. For the lateral nucleus, there were no differences in volume. This analysis pipeline will allow specific sites along the rostral-caudal axis of the LC to be identified for further neuronal analyses aimed at understanding the mechanisms responsible for LC vulnerability and its impacts on cognition in normative aging and disease. Furthermore, our data indicate that aging impacts distinct compartments of the LC uniquely.

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Poster

P894. Learning and Memory in Aging: Animal Models II

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Program #/Poster #: P894.10

Topic: H.12. Aging and Development

Support: McKnight Brain Research Foundation
State of Arizona – DHS
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TBIR7T
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Title: MRI micro- and macro-structural biomarkers linked to nonpathological brain aging in macaques

Authors: *L. DIECKHAUS[1], ***L. DIECKHAUS**[2], K. E. MCDERMOTT[1], A. MURLIKRISHNAN[3], E. M. FOIRYOLO[3], D. T. GRAY[1], E. B. HUTCHINSON[4], C. A. BARNES[1,5];
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Abstract: Imaging biomarkers that can distinguish normal aging processes from age-related pathology such as Alzheimer's Disease (AD) may improve clinical diagnosis and advanced microstructural MRI may provide novel markers. Because nonhuman primates provide an excellent model for studying non-pathological, or normative brain aging - i.e., they do not spontaneously develop AD - we performed high resolution *ex vivo* imaging on the perfused whole brains of 12 behaviorally characterized female bonnet macaques ranging from 10 to 25 years old (30 to 75 human equivalent years). Three different micro-structural MRI metrics were examined: fractional anisotropy (FA) from diffusion tensor imaging (DTI), myelin water fraction (MWF) from multi-echo T2 and bound pool fraction (BPF) from selective inversion recovery magnetization transfer MRI. Targeted assessment of metric values in the hippocampus and in white matter was performed by manual region of interest (ROI) analysis. Processing for DTI using the TORTOISE software package and for MWF and BPF using the REMMI MATLAB toolbox was executed on the University of Arizona's high-performance computing (HPC) clusters using MATLAB, Python, and bash scripts. High-resolution maps were calculated for each brain and manual ROI segmentation masks for the hippocampus and white matter were created manually using ITKsnap and FA, MWF and BPF metric values were extracted for these regions. Correspondence between these metrics as well as alterations associated with advanced age in the absence of overt pathology were assessed by Pearson's correlation. ROI analysis of the whole hippocampus and white matter did not reveal any statistically significant correlation with age from any of the MRI map metrics collected. A significant positive correlation (R= 0.688, p= 0.013) was observed between MWF and BPF for the hippocampus, but low correlation was observed between FA and MWF (R= 0.07, p= 0.82) and FA and BPF (R= 0.29, p= 0.36). For white matter, a significant positive correlation was observed between FA and MWF (R=0.72, p= 0.008) but low correlation was observed between FA and BPF (R= -0.14, p= 0.66) and MWF and BPF (R= 0.094, p= 0.77). This observation indicates that both MWF and BPF mapping provide information on white matter condition that is distinct from that provided by more traditional FA measures. Together, these data lay the foundation for a rigorous imaging pipeline that will provide novel foundational observations regarding multiple radiological changes that occur in nonpathological brain aging.

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Poster

P933. Optical Methods: Imaging Neural Activity and Behavioral Analysis

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Program #/Poster #: P933.01

Topic: I.04. Physiological Methods

Support: U.S. Department of Education Award Number P382A150041

Title: Connectomes of organelles in developing and adult mouse brains

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Abstract: Understanding the development of organelles is important. Large volume electron microscopy (LVEM) is an excellent technique that helps to examine the development of organelles because most membranes and structures are labeled with ocimum. In LVEM data sets of adult mouse brains we observe mitochondria and the nuclear pore clusters on the nuclear membrane in close proximity to one another. The nuclear envelope plays a significant role in protecting the genetic code, as well as enabling transport of the mRNA into the cytoplasm through the nuclear pores. It has been established that the in- and outward flow of mRNA occurs through the nuclear pores, however little is known about the inward transport of the energy molecules that fuel many processes. To examine the spatial relationship between the nuclear pores and the mitochondria we annotated two LVEM data sets - one from an adult mouse and one from a P14 mouse using VAST Lite software to outline these structures. The mitochondria were found and annotated using 3D Unet parallelized on Argonne supercomputers. Approximately 1000 serial sections were annotated altogether for comparison purposes. Lastly, the distance between randomly selected nuclear pore clusters and mitochondria will be calculated using an algorithmic formula in Matlab. In comparison with the scientific data on the proximity of mitochondria to the nuclear pores and our observational data, we hypothesize that the distance is strongly related to the cluster formation of the pores in the membrane. We conclude that LVEM has excellent potential to answer whether distance plays a role in the formation of the nuclear pore clusters.

Disclosures: S. Turbiarz: None. **G. Wildenberg:** None. **H. Li:** None. **N.B. Kasthuri:** None. **A. Maselli:** None.

Poster

P933. Optical Methods: Imaging Neural Activity and Behavioral Analysis

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P933.02

Topic: I.04. Physiological Methods

Support: NINDS UF1 NS116241

Title: Off-the-shelf Achromatic Objective Design for use with GRIN Lens

Authors: *M. D. YOUNG[1], E. A. GIBSON[3], D. RESTREPO[2];
[1]Bioengineering, [2]Cell & Dev. Biology, Neurosci. Program, Univ. of Colorado Anschutz Med. Campus, Aurora, CO; [3]Bioengineering, Univ. of Colorado Denver, Aurora, CO

Abstract: Gradient refractive index (GRIN) lenses have found a ubiquitous use in the field of neuroscience. GRIN lenses allow optical imaging of deeper brain regions with minimal tissue damage by achieving high numerical apertures with small diameter rod lens designs (Jung & Schnitzer, 2003).
GRIN rod lenses are typically monochromatic and are susceptible to chromatic aberration and are thus more suitable for monochromatic or near-monochromatic applications such as linear fluorescence microscopy. While there are commercially available GRIN rod lenses that accomplish the achromatic correction by using a doublet refractive lens joined to a GRIN rod lens, these lenses are prohibitively expensive. In addition, GRIN lenses are implanted in an animal and it is difficult to remove and clean for continuous use.
Here, we present an objective lens that uses commercial-off-the-shelf (COTS) lenses. This objective lens is designed in conjunction with a commercially available, inexpensive GRIN singlet lens and is adjusted to compensate for the chromatic aberration of the combined image system. Achromatic performance is evaluated at two wavelengths and secondary spectrum is not optimized. Design embodiments are presented for combinations of linear (1P) and multiphoton (2P) sources, e.g., 920 nm and 488 nm. This allows imaging at 1P and 2P wavelengths without adjustment of the image plane. The collection efficiency of epifluorescent light evaluated using nonsequential ray tracing (Beaurepaire & Mertz, 2002) (Zinter & Levene, 2011) (Oheim, Beaurepaire, Chaigneau, Mertz, & Charpak, 2001).
The design is compared against the monochromatic and polychromatic performance of the GRIN lens without an objective lens and with an objective lens from Thorlabs (TL10X-2P). A bill of materials for the lens components is provided.
In addition, we consider how field correction may be added in the future to improve irradiance across the field of view.
Beaurepaire, E., & Mertz, J. (2002). Appl. Opt., 41, 5376--5382.
Jung, J. C., & Schnitzer, M. J. (2003). Opt. Lett., 28, 902--904. doi:10.1364/OL.28.000902
Oheim, M., Beaurepaire, E., Chaigneau, E., Mertz, J., & Charpak, S. (2001). Journal of

Neuroscience Methods, 111(1), 29--37.
Zinter, J. P., & Levene, M. J. (2011). Opt. Express, 19, 15348--15362.

Disclosures: M.D. Young: None. **E.A. Gibson:** None. **D. Restrepo:** None.

Poster

P933. Optical Methods: Imaging Neural Activity and Behavioral Analysis

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Program #/Poster #: P933.03

Topic: I.04. Physiological Methods

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R01 MH120162
Botanical Center Pilot Award from P50 AT008661-01 from the NCCIH and the ODS (Pasinetti PI)
One Mind Otsuka Rising Star Award
McKnight Memory and Cognitive Disorders Award
Klingenstein-Simons Fellowship Award in Neuroscience
Mount Sinai Distinguished Scholar Award

Title: Minian an open-source miniscope analysis pipeline

Authors: *Z. DONG[1], W. MAU[1], Y. FENG[1], Z. T. PENNINGTON[1], L. CHEN[1], Y. ZAKI[1], K. RAJAN[1], T. SHUMAN[1], D. AHARONI[2], D. CAI[1];
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Abstract: Miniature microscopes have gained considerable traction for *in vivo* calcium imaging in freely behaving animals. However, extracting calcium signals from raw videos is a computationally complex problem and remains a bottleneck for many researchers utilizing single-photon *in vivo* calcium imaging. Despite the existence of many powerful analysis packages designed to detect and extract calcium dynamics, most have either key parameters that are hard-coded or insufficient step-by-step guidance and validations to help the users choose the best parameters. This makes it difficult to know whether the output is reliable and meets the assumptions necessary for proper analysis. Moreover, large memory demand is often a constraint for setting up these pipelines since it limits the choice of hardware to specialized computers. Given these difficulties, there is a need for a low memory demand, user-friendly tool offering interactive visualizations of how altering parameters at each step of the analysis affects data output. Our open-source analysis pipeline, Minian (Miniscope Analysis), facilitates the transparency and accessibility of single-photon calcium imaging analysis, permitting users with little computational experience to extract the location of cells and their corresponding calcium

traces and deconvolved neural activities. Minian contains interactive visualization tools for every step of the analysis, as well as detailed documentation and tips on parameter exploration. Furthermore, Minian has relatively small memory demands and can be run on a laptop, making it available to labs that do not have access to specialized computational hardware. Minian has been validated to reliably and robustly extract calcium events across different brain regions and from different cell types. In practice, Minian provides an open-source calcium imaging analysis pipeline with user-friendly interactive visualizations to explore parameters and validate results.

Disclosures: Z. Dong: None. **W. Mau:** None. **Y. Feng:** None. **Z.T. Pennington:** None. **L. Chen:** None. **Y. Zaki:** None. **K. Rajan:** None. **T. Shuman:** None. **D. Aharoni:** None. **D. Cai:** None.

Poster

P933. Optical Methods: Imaging Neural Activity and Behavioral Analysis

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Topic: I.04. Physiological Methods

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Botanical Center Pilot Award to DJC
One Mind Otsuka Rising Star Award to DJC

Title: ezTrack: an open-source video analysis pipeline for the investigation of animal behavior

Authors: *A. R. LABANCA, T. R. FRANCISCO, S. I. LAMSIFER, K. S. DIEGO, O. LIOBIMOVA, Z. T. PENNINGTON, D. J. CAI;
Icahn Sch. of Med. At Mount Sinai Grad. Training Program In Neurosci., New York, NY

Abstract: Tracking animal behavior by video is one of the most common tasks in neuroscience. Although commercial software exists for executing this task, they often present enormous costs to the researcher and can entail purchasing expensive hardware that lacks adaptability. In addition to this, tracking animal behavior often requires extensive experience working with code, making it challenging for those without a programming background. To address these issues, we created and validated ezTrack: an open-source, user-friendly pipeline that makes tracking small animal behavior easy for those with and without programming experience. ezTrack's two modules, the Location Tracking Module and Freeze Analysis Module, can be used for the positional analysis of an individual animal, amenable to a wide range of behavioral tasks, and to

analyze defensive freezing behavior respectively. For both modules, a range of interactive plots and visualizations are available to confirm that chosen parameters produce the anticipated results. Moreover, batch processing tools for rapid analysis of multiple videos are provided and frame-by-frame output makes alignment with biological recording data simple. We now present step-by-step instructions for the use of ezTrack, as well as various tips to make using the software as easy and efficient as possible. Additionally, we present updates made to ezTrack since its original publication, including a mechanism for defining regions of an arena to be excluded from analysis, a wire removal algorithm, and a downsampling feature.

Disclosures: A.R. LaBanca: None. **T.R. Francisco:** None. **S.I. Lamsifer:** None. **K.S. Diego:** None. **O. Liobimova:** None. **Z.T. Pennington:** None. **D.J. Cai:** None.

Poster

P933. Optical Methods: Imaging Neural Activity and Behavioral Analysis

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Program #/Poster #: P933.05

Topic: I.04. Physiological Methods

Support: K01MH103594
R21MH109775
R21NS098020

Title: Mapping cortical networks in essential tremor with deep brain stimulation using high-density diffuse optical tomography

Authors: *M. MUNSI[1], A. SHERAFATI[2], A. T. EGGEBRECHT[3], T. M. BURNS-YOCUM[5], H. M. LUGAR[6], A. NARAYANAN[9], T. DOTY[4], S. EISENSTEIN[4], A. M. SVOBODA[10], M. L. SCHROEDER[10], A. Z. SNYDER[7], M. USHE[4], J. P. CULVER[11], T. HERSHEY[8];
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Abstract: Deep-brain stimulation (DBS) can provide substantial clinical motor benefit to patients with Essential Tremor (ET), when implanted in the ventro-intermediate nucleus of the thalamus (VIM) [1]. Knowing the extent of DBS cortical effects on functional connectivity (FC) are essential for enhancing future DBS implantations, stimulation delivery, and optimization procedures. However, the cortical impacts of DBS and its potential side-effects are difficult to study using conventional neuroimaging tools such as Positron Emission Tomography (PET) due

to radiation exposure and limited temporal resolution, and functional Magnetic Resonance Imaging (fMRI) due to safety concerns from contraindications. To address these limitations, we used a previously validated High Density Diffuse Optical Tomography (HD-DOT) system with 96 sources and 92 detectors [2] to understand the neural mechanisms underlying the cortical effects of VIM DBS in ET patients. Ten ET patients (2f; ages 53-70) and 11 age-matched controls (8f; ages 52-71) were scanned using this HD-DOT device during resting state, auditory, and visual tasks. The control group underwent an fMRI scan with the same protocol. We demonstrated the feasibility and sensitivity of HD-DOT in imaging controls and ET patients with VIM DBS ON and OFF using an established data analysis pipeline [3]. Task activations and resting state networks in ET patients anatomically co-aligned with the data collected from the controls. These preliminary results validate the feasibility and resolution of HD-DOT in accurately mapping task responses and FC within the ET population (Figures 1 and 2). In the future, we aim to evaluate network connectivity differences between ET and control cohorts and between on/off conditions. **References:** [1] Gallea C, et al. *Brain,* 2015. [2] Eggebrecht AT, et al. *Nat Photonics,* 2014. [3] Sherafati A, et al. *Hum. Brain Mapping*, 2020.

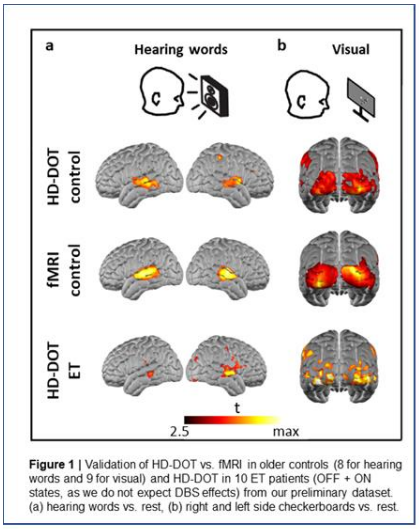


Figure 1 | Validation of HD-DOT vs. fMRI in older controls (8 for hearing words and 9 for visual) and HD-DOT in 10 ET patients (OFF + ON states, as we do not expect DBS effects) from our preliminary dataset. (a) hearing words vs. rest, (b) right and left side checkerboards vs. rest.

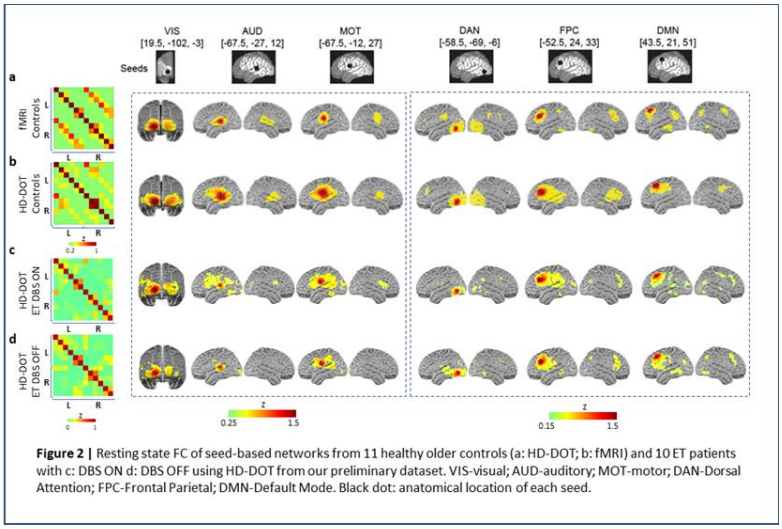


Figure 2 | Resting state FC of seed-based networks from 11 healthy older controls (a: HD-DOT; b: fMRI) and 10 ET patients with c: DBS ON d: DBS OFF using HD-DOT from our preliminary dataset. VIS-visual; AUD-auditory; MOT-motor; DAN-Dorsal Attention; FPC-Frontal Parietal; DMN-Default Mode. Black dot: anatomical location of each seed.

Disclosures: M. Munsi: None. **A. Sherafati:** None. **A.T. Eggebrecht:** None. **T.M. Burns-Yocum:** None. **H.M. Lugar:** None. **A. Narayanan:** None. **T. Doty:** None. **S. Eisenstein:** None. **A.M. Svoboda:** None. **M.L. Schroeder:** None. **A.Z. Snyder:** None. **M. Ushe:** None. **J.P. Culver:** None. **T. Hershey:** None.

Poster

P933. Optical Methods: Imaging Neural Activity and Behavioral Analysis

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P933.06

Topic: I.04. Physiological Methods

Support: DARPA N66001-17-C-4012
NSF 1706207

Title: An Implantable, Mechanically Flexible Neural Interface for All-Optical Fluorescence Imaging and Optogenetic Stimulation

Authors: *E. H. POLLMANN, H. YIN, K. L. SHEPARD;
Columbia Univ., New York, NY

Abstract: Understanding the human brain is one of the greatest scientific challenges. Despite many advancements in brain-computer interfaces (BCI) during the last few decades, there is still little understanding of neuron-level connectivity in brain computations and information processing. Recently, optical techniques have gained popularity over electrophysiological techniques because of the ability to record in-vivo (using genetically encoded calcium indicators) and stimulate (using optogenetic indicators) neural activity at the single cell-level with action-potential sensitivity. However, state-of-the-art fluorescence microscopes are expensive, bulky, and stationary and do not allow for free-behaving animal studies. In the current state-of-the-art, a variety of head-mounted miniature microscopes have been demonstrated; however, the bulky lenses preclude full implantation. High selectivity for optoelectronic stimulation available through the use of, for example, spatial light modulators with free-space optics is also largely unavailable in this form factor. We have been exploring fully implantable optoelectronics devices, imagers, and light-emitting diodes, by exploiting advances in complementary metal-oxide-semiconductor (CMOS) microelectronics and heterogeneous package integration. Here, we show one such form factor, a ~150-μm-thick, mechanical flexible form-factor that can conform to the surface of the brain. The device consists of an array of 160×160 time-gated monolithic single-photon avalanche photodiodes (SPAD) detectors low-light-intensity imaging and dual color (blue and green) flip-chip bonded micro-LEDs (μLED) as light sources both for optogenetic stimulation and fluorescence excitation. For fluorescence imaging, we co-optimized a hybrid absorption/interference filter, which is deposited and packaged directly on the surface of the imager, along with the μLED arrays to reject the 470-nm blue excitation light in favor of 520-nm green emission with an optical density (OD) exceeding six. Furthermore, we leverage computational imaging techniques used to obtain large field-of-view (FoV), high-resolution images without the use of focusing lenses. The CMOS chip is die-thinned to approximately ~7.5μm to be mechanically flexible, and the chip is packaged on a flexible printed circuit board. This fully implantable camera displaces an implant volume of less than $10mm^3$ while implementing all the components of fluorescence-based neural interface device.

Disclosures: E.H. Pollmann: None. **H. Yin:** None. **K.L. Shepard:** None.

Poster

P933. Optical Methods: Imaging Neural Activity and Behavioral Analysis

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Topic: I.04. Physiological Methods

Support: 1700408 Neurotech Hub
NIH BRAIN UF1NS107668 (MAB and PG)

Title: The Miniscope Project: an open-source neural imaging platform for freely behaving animals

Authors: *F. SANGIULIANO JIMKA[1], G. CHANGLIANG[1], R. CHANG[1], P. ZHAO[2], M. SEHGAL[3], G. BLAIR[4], H. T. BLAIR, IV[5], M. A. BASSO[6], A. J. SILVA[7], P. GOLSHANI[8], D. AHARONI[2];
[2]Dept. of Neurol., [3]Neurobio., [4]Psychology, [5]Dept Psychology, [6]Psychiatry and Biobehavioral Sci. and Neurobio., [1]UCLA, Los Angeles, CA; [7]Dept Neurobiol, UCLA Med. Ctr., Los Angeles, CA; [8]UCLA Dept. of Neurol., Los Angeles, CA

Abstract: In recent years, miniaturized fluorescence microscopes (miniscopes) have taken a central role in behavioral neuroscience due to their reduced size and weight. When used in conjunction with genetically encoded calcium indicators (GECIs) these head-mounted lightweight imaging devices have enabled researchers to study the dynamics of neural populations with single cell resolution in freely behaving animals. The goal of the open-source UCLA Miniscope Project is to advance the field of neural imaging in freely behaving animals through continued open-source development and dissemination of new tools and techniques. Here we present the current state of the UCLA Miniscope Project, covering a comprehensive set of open-source devices and data acquisition (DAQ) hardware/software under development and recently released. This work is aimed at democratizing access to transformative tools while also advancing, integrating, and streamlining neuro-behavioral research. These devices include our newest generation of epifluorescence Miniscopes capable of electronic focusing, cranial window imaging, and single cell resolution across greater than a 1mm diameter field-of-view (FOV). Building off this new generation of Miniscopes, we also have developed dual channel systems for sequential imaging or imaging plus optogenetics, electrophysiology integrated Miniscopes, and wire-free Miniscopes. In addition, we have developed a large FOV Miniscope for rats and larger animals capable of single cell resolution imaging at video frame rates across a 3.2mm x 2.5mm FOV. In order to streamline the integration of neural and behavioral data, we have developed a new open-source behavioral camera, MiniCAM, with a 5MP image sensor, interchangeable lenses, and a nIR LED illumination ring. All devices share common modular open-source DAQ hardware and software. The software handles the connection, control, visualization, streaming, and data logging of many devices simultaneously. It includes a real-time dF/F display, GPU accelerated data visualizations, and built-in pose estimation using

embedded Python and Deeplabcut-Live. Open-source UCLA Miniscope devices have been adopted into hundreds of labs across over 18 countries and have laid the foundation for novel modifications and advancements by many other groups. Through open-source development and engagement with the scientific community we hope to continue addressing economic and technical hurdles and broaden access to these technologies.

Disclosures: F. Sangiuliano Jimka: None. **G. Changliang:** None. **R. Chang:** None. **P. Zhao:** None. **M. Sehgal:** None. **G. Blair:** None. **H.T. Blair:** None. **M.A. Basso:** None. **A.J. Silva:** None. **P. Golshani:** None. **D. Aharoni:** None.

Poster

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Program #/Poster #: P933.08

Topic: I.04. Physiological Methods

Title: Imaging genetically encoded neurotransmitter sensors using head-mountable miniscopes

Authors: K. ZITELLI[1], J. ZAPATA[1], D. HOUTTEMAN[2], P. BONNAVION[2], A. DE KERCHOVE D'EXAERDE[2], *A. STAMATAKIS[1];
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Abstract: Genetically encoded dynamic fluorescent sensors, such as calcium and voltage sensors, are powerful tools for monitoring neural activity. Recently developed neurotransmitter sensors represent a promising addition to this family of sensors. To date, genetically encoded neurotransmitter sensors have been imaged primarily in vivo with fiber photometry, which lacks spatial resolution. In vivo 2-photon microscopy offers high spatial resolution, but requires head fixation, limiting the free behavior of the test subject. Miniscopes such as the nVista^{TM} system have been used to image genetically encoded sensors such as GCaMP, allowing for free behavior while monitoring neural activity with high spatial resolution. Here, we demonstrate the feasibility of imaging genetically encoded neurotransmitter sensors during free behavior using Inscopix miniscopes. Mice were injected with the GPCR-activation-based dopamine sensor $GRAB_{DA}$ in the dorsal striatum or nucleus accumbens (NAc, Shell), and with the opsin ChrimsonR in the substantia nigra or ventral tegmental area (VTA), respectively. Using the optogenetically-enabled miniscope nVokeTM system, $GRAB_{DA}$ activity was monitored as mice explored an open field. Dopamine release was elicited from nigral or dopaminergic VTA fibers via stimulation of ChrimsonR, mediating a significant increase in $GRAB_{DA}$ signal in both the dorsal striatum and NAc. In another experiment, amphetamine (4 mg/kg) administered to mice led to a marked increase in $GRAB_{DA}$ signal in both brain areas. These findings demonstrate that miniscopes are an excellent tool for imaging genetically encoded neurotransmitter sensors, and pave the way forward for taking full advantage of such imaging platforms, including leveraging

the multiple channels and spatial resolution of the nVueTM system to simultaneously image cellular-resolution GCaMP activity alongside red neurotransmitter sensors.

Disclosures: K. Zitelli: A. Employment/Salary (full or part-time):; Inscopix. **J. Zapata:** A. Employment/Salary (full or part-time):; Inscopix. **D. Houtteman:** None. **P. Bonnavion:** None. **A. De Kerchove D'Exaerde:** None. **A. Stamatakis:** A. Employment/Salary (full or part-time):; Inscopix.

Poster

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Program #/Poster #: P933.09

Topic: I.04. Physiological Methods

Support: ANR-15-CE19-0011-01
ANR-17-CE37-0022
DIM Cerveau & Pensée, ALPINS
Fondation pour l'Audition (FPA RD-2018-6)
ANR-17-CE37-02
ANR-19-CE37-0016

Title: Highly stable head-holders for experiments in head-fixed animals

Authors: J. FODOR[1], J.-F. LEGER[2], ***L. BOURDIEU**[3], C. VENTALON[2];
[1]Ymetry, Bagnolet, France; [2]CNRS - Ecole Normale Superieure, PSL, Paris, France; [3]École Normale Supérieure, Paris, France

Abstract: The past fifteen years have been marked by spectacular progresses in the field of integrative neurophysiology, underlid by technical innovations enabling the study of neuronal processes in behaving animals. In particular, recordings in head-fixed animals (mice, rats or ferrets) have provided original information that have revolutionized our understanding of the cellular and network mechanisms underlying sensory processing, motor planning, memory and learning and higher order cognitive processes. To perform such experiments, the animal needs to carry a miniaturized head holder that allows easy fixation of its head to the recording apparatus. These holders should be light enough to be carried easily by the animals in their cage. They should provide optimal stability with sufficient access to the region of interest. They should be compatible with providing unrestricted sensory inputs (e.g. visual or tactile inputs) to the animal. For chronic recordings they should allow recording with a high positional repeatability. They should also provide the possibility to integrate multiple measurement systems on the same animal as for example 2-photon microscopy for optical recording and/or perturbation of neuronal activity using optogenetic tools, optical fibers for photometry or photoactivation / inhibition,

electrophysiological systems (EEG, EMG, LFP or unit activity), drug delivery or electrical micro-stimulation systems. Current commercially-available solutions only satisfy a set of well-defined and standard experimental situations. They are mainly limited to large and flat head plates that can strongly hindered sensory inputs and do not integrate additional measurements. Using 3D-printing in titanium, we have developed innovative custom head-holders based on an original design that minimizes weight and perturbations to the animal behavior and maximizes mechanical stability and experimental repeatability. They benefit from the use of Titanium and of a 3D design that provide uniquely both rigidity and lightness at low cost. Several different prototypes have already been designed, produced and used. Thanks to their design methods, novel head-holders can be adapted to different animal models, brain areas and to integrate multiple measurement / perturbation systems.

Disclosures: J. Fodor: A. Employment/Salary (full or part-time):; Ymetry. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent holder. **J. Leger:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent holder. **L. Bourdieu:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent holder. **C. Ventalon:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); patent holder.

Poster

P956. Network Models: Experimentation: Cortex

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P956.01

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

Support: NIH RO1EB022889

Title: HNN-core: an open source Pythonic interface to the Human Neocortical Neurosolver (HNN) software for cellular and network interpretation of human MEG and EEG signals

Authors: *M. JAS[1], *N. TOLLEY[2], C. BAILEY[4], B. CALDWELL[2], *R. THORPE[3], K. LOI[2], M. HAMALAINEN[1], S. R. JONES[2];
[1]Massachusetts Gen. Hosp., Charlestown, MA; [3]Engin., [2]Brown Univ., Providence, RI; [4]Aarhus Univ., Aarhus, Denmark

Abstract: The Human Neocortical Neurosolver software (HNN, https://hnn.brown.edu) is a neural modeling software developed to understand and interpret the cellular and circuit neural underpinnings of human magneto-/electro-encephalography (M/EEG) signals. The foundation of HNN is a biophysically-detailed neocortical model along with workflows to interact with this

model through layer-specific synaptic activation patterns to simulate the most commonly measured M/EEG signals including event related potentials (ERPs) and low-frequency brain rhythms. HNN-core (https://jonescompneurolab.github.io/hnn-core) is a new lightweight library that provides a Pythonic interface to the HNN functionality for simulating macroscale human M/EEG signals. HNN-core is designed to simulate primary electrical current dipole time courses and compare directly against source-localized MEG and EEG data in physical units (Ampere-meters). HNN-core is distributed with examples for simulating brain rhythms following the workflows in the HNN tutorials: https://HNN.brown.edu/tutorials.
The goal of HNN-core is complementary to HNN. Its object-oriented design allows the computational neuroscience community to understand and contribute to the development and use of the HNN software suite, following best practices in open-source software. The Python Application Programming Interface (API) allows easy batch processing and integration with other Python based packages, such as MNE-Python. Furthermore, the HNN-core API is designed to promote a community-driven software development effort. The package is unit tested with 90% code coverage and new changes are checked using continuous integration tests to avoid regression bugs. The codebase adheres to modern Python style conventions and is extensively documented using function docstrings. The first version of HNN-core is available to install on PyPi. Here, we will provide examples of using HNN-core, including new features such as the ability to insert recording electrodes in the extracellular medium to measure Local Field Potentials (LFPs), functions to modify local network connectivity, and template network models to reproduce published results. We are currently working on optimization of model parameters to match source-localized data and a lightweight GUI interface to HNN-core using IPywidgets, designed to mimic the functionality in the current HNN GUI. Future work will focus on incorporation of layer-resolved LFP and current source density plots, parameter sensitivity analysis and uncertainty estimation of learned parameters, and interoperability with existing tools such as Netpyne, NeuroML and LFPy.

Disclosures: M. Jas: None. **N. Tolley:** None. **C. Bailey:** None. **B. Caldwell:** None. **R. Thorpe:** None. **K. Loi:** None. **M. Hamalainen:** None. **S.R. Jones:** None.

Poster

P956. Network Models: Experimentation: Cortex

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Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P956.02

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

Support: McKnight Doctoral Fellowship - Florida Education Fund
CTSI-Pilot-FY2021-04
University of Miami

Title: Laminar specificity of the auditory awareness negativity under multitone masking: a biophysical modeling study

Authors: C. FERNANDEZ PUJOL, E. G. BLUNDON, *A. R. DYKSTRA;
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Abstract: Electroencephalography (EEG) and magnetoencephalography (MEG) are excellent mediums for capturing human neural activity on a millisecond time scale, yet little is known about their underlying laminar and biophysical basis. Here, we used a reduced but realistic cortical circuit model - Human Neocortical Neurosolver (HNN) - to shed light on the laminar specificity of brain responses associated with auditory conscious perception under multitone masking. HNN provides a canonical model of a neocortical column circuit, including both excitatory pyramidal and inhibitory basket neurons in layers II/III and layer V. We found that the difference in event-related responses between perceived and unperceived target tones could be accounted for by additional input to supragranular layers arriving from either the non-lemniscal thalamus or cortico-cortical feedback connections. Layer-specific spiking activity of the circuit revealed that the additional negative-going peak that was present for detected but not undetected target tones was accompanied by increased firing of layer-V pyramidal neurons. These results are consistent with current cellular models of conscious processing and help bridge the gap between the macro and micro levels of analysis of perception-related brain activity.

Disclosures: C. Fernandez Pujol: None. **E.G. Blundon:** None. **A.R. Dykstra:** None.

Poster

P956. Network Models: Experimentation: Cortex

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P956.03

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

Support: NIMH Grant R21MH125199

Title: Neocortical microcircuit changes underlying neocortical plasticity: a computational study

Authors: *M. Z. KHALIL[1], P. SKOSNIK[2], M. A. SHERIF[3];
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Abstract: Neocortical plasticity plays an essential role in multiple psychiatric disorders, including major depressive disorder (MDD), PTSD, and schizophrenia. Long-term potentiation (LTP) is the hallmark of cortical synaptic plasticity in animal models, mediated by the insertion of AMPA receptors (AMPARs) into postsynaptic membranes following tetanic stimulation.

Assessing neocortical plasticity directly in humans has been possible using an EEG-LTP task [1,2,3,4,5]. In the task, sensory event-related potentials (ERPs) are recorded before and after tetanic stimulation in the auditory or visual modalities. The LTP marker in the task is an increase in the absolute amplitude of the N100 ERP component following the tetanic stimulation. Understanding the microcircuit changes underlying the LTP marker in humans will allow us to infer neocortical microcircuit abnormalities in patients suffering from brain disorders affecting plasticity. To examine if the increase in N100 amplitude could be reproduced by increasing AMPA receptors density in the postsynaptic terminals in neocortical columns, we used the Human Neocortical Neurosolver (HNN), a validated neocortical microcircuit model for the EEG/MEG dipole [6,7]. HNN consists of 100 multicompartmental layer 2/3 and 100 layer 5 pyramidal neurons, implemented using the simulation platform NEURON, with fast-spiking interneurons in both layers. HNN simulates the ERP waveform using thalamocortical and cortico-cortical inputs to the model. We ran simulations to examine increasing the AMPAR density on the distal apical dendrites of layers 2/3 and layer 5 pyramidal neurons and on the interneurons in layer 2/3. Doubling the AMPAR density at these locations did not change N100 amplitude. So we ran another set of simulations to examine if augmenting AMPARs on postsynaptic terminals sharing a common input source, as reflected by input synchronization in the cortico-cortical inputs, would replicate the LTP marker. Interestingly, synchronizing the input to the target cells increased the absolute N100 amplitude by 30%. Thus, tetanic stimulation in the EEG-LTP task seems to potentiate synapses receiving inputs from a common source. Our results provide specific predictions about the cortico-cortical connectivity in the EEG-LTP task. Combining EEG tasks and modeling hold the potential to infer microcircuit changes in patients struggling with disorders like MDD, where cortical plasticity and dendritic functioning are impaired.

Disclosures: M.Z. Khalil: None. **P. Skosnik:** None. **M.A. Sherif:** F. Consulting Fees (e.g., advisory boards); In Silico Biosciences, Inc..

Poster

P956. Network Models: Experimentation: Cortex

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:00 PM - 2:00 PM

Program #/Poster #: P956.04

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

Title: Multi-scale dynamical modeling for spike and LFP activity using a NeuroBondGraph Network

Authors: *Y.-J. CHANG[1], Y.-I. CHEN[1], H.-C. YEH[1], S. R. SANTACRUZ[1,2,3];
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Abstract: The brain exhibits computational structure across a variety of scales: from single neurons (micro-scale) to functional areas (meso-scale) to cortical networks (macro-scale). In neuropsychiatric conditions, pathological activity is typically neural circuit-wide, impacting dynamics at multiple scales, either directly or indirectly. Combining information on changes in brain dynamics at multiple scales can shed new light on the biological cause of neuropsychiatric disorders. As neural dynamics illuminate the causal interactions between neurons or networks, it is necessary to develop a neural dynamics-based framework to assess the brain computations governing cognitive functions. While single-level neural dynamics encode rich information giving rise to behavior, the different levels of brain network can communicate with each other. However, *how* the activity and information at one level interact with other levels is seldom explored. Evidenced by the success of cross-scale modeling between local field potentials (LFP) and signals recorded from screw type electrodes implanted in the skull via the NeuroBondGraph Network (NBGNet) in the previous work, we develop the multi-scale NBGNet to study neural communications between spike and LFP in the brain networks. Neural representation extracted from multi-scale NBGNet can potentially explain the naturalistic behaviors, which will be validated in real-time brain-machine interface experiments. With the incorporation of realistic brain constraints in the multi-scale NBGNet, this neurobiologically realistic framework holds great potentials to improving our understanding of complex brain functions in unprecedented detail.

Disclosures: Y. Chang: None. **Y. Chen:** None. **H. Yeh:** None. **S.R. Santacruz:** None.

Poster

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Program #/Poster #: P956.05

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

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Title: Simulation based inference in the Human Neocortical Neurosolver neural modeling software uncovers circuit mechanisms of primate motor cortical 15-29 Hz beta activity during motor planning

Authors: *N. TOLLEY[1], P. L. RODRIGUES[2], A. GRAMFORT[2], J. DONOGHUE[1], C. VARGAS-IRWIN[1], S. R. JONES[1];
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Abstract: Beta frequency (~15-29 Hz) activity in the neocortex has a well documented association with sensorimotor information processing, however, its circuit level generation remains poorly understood. We employed the Human Neocortical Neurosolver (HNN), a framework whose foundation is a cortical column model with cell and circuit level detail designed to connect macroscale signals to meso/microcircuit level phenomena (Neymotin 2020). This model was applied to study the cellular and circuit mechanisms of beta generation using local field potential (LFP) recordings from the non-human primate (NHP) motor cortex.
To characterize beta producing mechanisms, we employed simulation based inference (SBI) in the HNN modeling tool. SBI is a framework that leverages machine learning techniques and neural density estimators to characterize the relationship between a large space of model parameters and simulation output. In this setting, Bayesian inference can be applied to models with intractable likelihood functions (Gonçalves 2020, Papamakarios 2021).
To predict neural mechanisms underlying beta activity with SBI in HNN, we utilized multi-electrode array neural recordings from NHP's performing an instructed delay reach-to-grasp task. Motor cortical LFP beta activity presented as transient 50 ms events with a stereotyped waveform shape, consistent with prior studies (Sherman 2016; Bonaiuto biorxiv 2021). Further analysis demonstrated a significant decrease in beta event amplitude after behavioral cues, consistent with event related desynchronization.
Our previous modeling studies have suggested that beta events emerge from coincident layer specific excitatory inputs that contact the proximal and distal dendrites of layer 5 cortical pyramidal neurons. These predictions were supported by laminar recordings in mice and NHPs (Sherman 2016), and more recently with high density human magnetoencephalography (Bonaiuto biorxiv 2021). Building from this prior work, we applied SBI in HNN to features of NHP LFP beta events and revealed the novel prediction that the timing between proximal and distal synaptic drives can account for changes in beta event amplitude.
Preliminary analysis of NHP data suggests that beta activity plays a strong modulatory role over spiking activity. Increased beta activity was associated with a reconfiguration of single unit activity into discrete nodes with more stereotyped firing patterns indicative of increased computational similarity (Hynes biorxiv 2019). We are currently working to incorporate these observations into our HNN inference framework to provide functional predictions for beta regulated information processing.

Disclosures: N. Tolley: None. **P.L. Rodrigues:** None. **A. Gramfort:** None. **J. Donoghue:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Beacon Biosignals. F. Consulting Fees (e.g., advisory boards); Otsuka Medical Devices, Beacon Biosignals. **C. Vargas-Irwin:** None. **S.R. Jones:** None.

Poster

P956. Network Models: Experimentation: Cortex

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

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Providence VA CfNN
Carney Institute for Brain Science
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Title: Expansion of the Human Neocortical Neurosolver neural molding tool to validate and inform modeling predictions with local field potential and current source density recordings

Authors: *A. ADEWAKUN[1,2], S. HAEGENS[3], M. N. O'CONNELL[4], T. M. MCGINNIS[5], A. BARCZAK[5], P. LAKATOS[5], C. E. SCHROEDER[3,5], S. A. NEYMOTIN[5], M. JAS[6], C. BAILEY[7], N. TOLLEY[8,9], R. THORPE[8,9], B. CALDWELL[8,9], S. R. JONES[8,9,10];
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Abstract: Magneto- and electro-encephalography (MEG/EEG) recordings allow scientists, neurologists and psychiatrists to non-invasively record brain activity from human subjects with a relatively fast temporal resolution. These powerful datastreams help uncover human information processing and diagnose neuropathologies, as well responses to therapeutics. However, the neural origins of these macroscale recordings are difficult to interpret, hindering their translational relevance. The Human Neocortical Neurosolver (HNN) neural modeling software tool was created to help researchers and clinicians develop and test hypothesis on the cellular and circuit level origin of M/EEG recordings (Neymotin et al eLife 2020; https://hnn.brown.edu). This project adds a new capability to HNN to simulate laminar local field potential (LFP) and the associated current source density (CSD), which are necessary to validate and inform HNN predictions with laminar recordings from animal models. We describe how the HNN modeling framework can be applied to simulate LFP and CSD by using line source approximations (LSA) and point source approximations (PSA) and new methods being developed to compare simulation results to empirical data from non-human primates (NHP). Our group is developing novel methods to extract features from simulated and experimental data representing canonical patterns of cortical activation in sensory event-related potentials, and to compare results with different temporal and spatial scales across species. Here, we focus on somatosensory evoked responses in humans and NHPs. This critical HNN expansion provides a new way to validate and inform HNN predictions about multi-scale laminar activity in the human neocortex by drawing on data collected from microelectrode recordings in animals.
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Poster

P956. Network Models: Experimentation: Cortex

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Program #/Poster #: P956.07

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

Support: Improvement of internationalization in the field of research and development at Charles University (CZ.02.2.69/0.0/0.0/17_050/0008466)
European Union Marie Skłodowska-Curie grant agreement No 861423 (enTRAIN vision)

Title: The impact of light propagation, neural morphology and network effects on the effective spatial resolution of optogenetic stimulation

Authors: *D. BERLING, L. BARONI, J. ANTOLÍK;
Fac. of Mathematics and Physics, Charles Univ., Prague, Czech Republic

Abstract: Enabling neuron-type specific stimulation and a higher biocompatibility than electrode-based approaches, optogenetic stimulation has become a powerful tool for studying the brain with a clinical application in neural prosthetic systems. Major experimental research effort is being invested into high resolution stimulators with focused light profiles [1] and methods to express light-sensitive channels at subcellular precision [2]. However, the combined effect of light propagation in cortical tissue, the distribution of light-sensitive channels across the neuron morphology, and the contribution of network effects onto the effective spatial resolution of the stimulation remains unclear. Recent biophysically accurate single neuron models which simulate the full neuron morphology under optical stimulation [3] and large-scale point-neuron network models with functionally specific cortical circuitry [4] partially address this open issue. However, the contribution of neural morphology in the context of functionally specific recurrent cortical networks remains unexplored, due to the challenge of simulating a large quantity of biophysically accurate single cell models.
Here, we address this open problem through a hybrid approach. Using data from a multi-compartment model of a cat V1 L2/3 pyramidal neuron [adapted from 3] we have trained a deep-neural network (DNN) to rapidly predict the light-induced currents into the soma for various illumination conditions. We then use these DNN-predicted currents as an input into a point-neuron network model of cat V1 to model the network behavior induced by optical stimulation [4].

The resulting simulation framework allows for the testing of different optogenetic transfection strategies, e.g. expression of channels preferentially at the soma or throughout the cell [2], as well as predicting the key characteristics of evoked activity for different specifications of cortical optogenetic stimulation setups.

References

1. Soltan, A et al. (2017). High Density, High Radiance μ LED Matrix for Optogenetic Retinal Prostheses and Planar Neural Stimulation. IEEE Trans Biomed Circuits Syst, 11(2), 347-359.
2. Shemesh, OA et al. (2017). Temporally precise single-cell-resolution optogenetics. Nat Neurosci, 20(12), 1796-1806.
3. Foutz, TJ, Arlow, RL, & McIntyre, CC (2012). Theoretical principles underlying optical stimulation of a channelrhodopsin-2 positive pyramidal neuron. J Neurophysiol, 107(12), 3235-3245.
4. Antolik, J et al. (2021). Assessment of optogenetically-driven strategies for prosthetic restoration of cortical vision in large-scale neural simulation of V1. Sci Rep, 11(1), 10783.

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Poster

P956. Network Models: Experimentation: Cortex

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Program #/Poster #: P956.08

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

Support: HiBALL Grant
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CIHR FDN-154298

Title: Long-range functional connectivity topographies parallel microstructural and cognitive hierarchies in the human brain

Authors: *Y. WANG[1], J. ROYER[1], B.-Y. PARK[1], R. VOS DE WAEL[1], S. LARIVIERE[1], C. PAQUOLA[1], B. MISIC[1], D. S. MARGULIES[2], J. SMALLWOOD[3], S. VALK[4], A. EVANS[1], B. BERNHARDT[1];
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Abstract: A key feature of human brain organization are long-range connections that mediate the communication across distributed networks in a massively expanded cortex. Here we profiled functional connectivity distance across the cortical mantle and assessed associations to cortical

microcircuity and cognitive taxonomies. We studied two datasets (50 healthy adults from our local acquisitions, 325 unrelated healthy adults from the HCP dataset) who underwent multimodal magnetic resonance imaging (MRI). In those individuals we combined cortex-wide functional MRI connectivity analysis and geodesic distance mapping, to profile a region's embedding within short- versus long-range functional networks. These functional connectivity distance computations were associated with the mapping of microstructural gradients based on quantitative T1 relaxometry in the same individuals, and post-mortem cytoarchitecture derived from BigBrain, as well as gene expression analysis derived from Allen Human Brain Atlas. Moreover, we utilized Neurosynth to decode gradients of functional connectivity distance, to examine associations to cognitive ontologies. Spatial correlation analysis employed non-parametric spin tests, to control for autocorrelations. We observed reproducible topographies of functional connectivity distance in both datasets running from sensory/motor areas with short-range connectivity towards transmodal systems such as the default mode network that are characterized by long-range connectivity. Spatial variations in connectivity distance were correlated with microstructural gradients based on in vivo MRI ($r = 0.75$, $p < 0.001$), as well as post-mortem cytoarchitecture ($r = 0.35$, $p < 0.001$). Similar associations were observed with respect to the principal component of gene co-expression, and gene enrichment indicated layer and cell specific genetic signatures of long-range connectivity. Finally, meta-analytical decoding revealed that higher-order functional terms such as 'social cognition' and 'reward' were associated with longer-range connectivity, while regions with shorter connectivity distance related to sensory/motor functions. Our findings provide novel insights into the correspondence of microstructural, functional, and cognitive hierarchies in the human brain.

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Poster

P956. Network Models: Experimentation: Cortex

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Program #/Poster #: P956.09

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

Support: Krembil Foundation
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Labatt Family Network

Title: Mapping inter-individual functional connectivity variability in TMS targets for major depressive disorder

Authors: *S. HARITA[1,4], D. MOMI[4], F. MAZZA[2,4], J. D. GRIFFITHS[3,4];
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Abstract: Background: Transcranial magnetic stimulation (TMS) is an emerging alternative to existing treatments for major depressive disorder (MDD). However, the effects of TMS on brain physiology and therapeutic outcomes are known to be highly variable from subject to subject. Proposed reasons for this variability include individual differences in neurophysiology, in cortical geometry, and in brain connectivity. Standard approaches to TMS target site definition tend to focus on individual brain regions implicated in MDD, such as the dorsolateral prefrontal cortex (dlPFC) and orbitofrontal cortex (OFC). Additionally considering the functional connectivity (FC) of these sites has the potential to improve subject-specificity of TMS targeting and, in turn, improve treatment outcomes. Objective & Hypothesis: We studied the FC of dlPFC and OFC TMS targets, based on induced electrical field (E-field) maps. We hypothesized that individual differences in spontaneous brain FC would contribute more to downstream network engagement than individual differences in cortical geometry (E-field variability). Methods: Tetrahedral head models were generated from T1-weighted MR images for 12 subjects from the Human Connectome Project database, and used to generate individualized E-field maps on the cortical surface for each subject. F3 and Fp1 electrode positions were used to target the left dlPFC and left OFC, respectively. We analyzed inter-subject variability in the shape and location of these TMS target E-field patterns, their FC, and the major functional networks to which they belong. Results: We observed key differences in TMS target FC between the dlPFC and OFC, and how this FC varied across subjects. Three major functional networks were targeted across the dlPFC and OFC: ventral attention, fronto-parietal (FPN) and default-mode (DMN) networks in the dlPFC, and the FPN and DMN in the OFC. Inter-subject variability in cortical geometry and FC was high. Our analyses showed that use of group-average subject E-field and FC allow prediction of which networks are targeted, but fail to accurately quantify the relative loading of TMS targeting on each of the principal networks. Conclusions: Based on our results, the high inter-individual variability in cortical geometry and FC, leading to high variability in distributions of targeted brain networks, may account for the high levels of variability in physiological and therapeutic TMS outcomes. We hope these insights prove useful as part of the broader effort by the psychiatry, neurology, and neuroimaging communities to help improve TMS therapy, through a better understanding of the technology and its neurophysiological effects.

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Poster

P053. Rett Syndrome II

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Program #/Poster #: P053.01

Topic: A.07. Developmental Disorders

Support: NIH Grant 1R01NS106285
International Rett Syndrome Foundation Grant #3064

Title: Vitamin D modulates cortical transcriptome and behavioral phenotypes in Mecp2 heterozygous Rett syndrome model mice

Authors: M. C. RIBEIRO, A. SINHA, ***J. L. MACDONALD**;
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Abstract: Rett syndrome (RTT) is an X-linked neurodevelopmental disorder caused by mutations in the transcriptional regulator *MECP2* (*Mecp2* in mouse). *Mecp2* loss-of-function leads to the disruption of many cellular pathways, including aberrant activation of the NF-κB pathway. Genetically attenuating the NF-κB pathway in *Mecp2*-null mice ameliorates hallmark phenotypes of RTT, including reduced dendritic complexity, raising the question of whether NF-κB pathway inhibitors could provide a therapeutic avenue for RTT. Vitamin D is a known inhibitor of NF-κB signaling; further, vitamin D deficiency is prevalent in RTT patients and male *Mecp2*-null mice. We previously demonstrated that vitamin D rescues the aberrant NF-κB activity and reduced neurite outgrowth of *Mecp2*-knockdown cortical neurons *in vitro*, and that dietary vitamin D supplementation rescues decreased dendritic complexity and soma size of neocortical projection neurons in both male hemizygous *Mecp2*-null and female heterozygous mice *in vivo*. Here, we have identified over 200 genes whose dysregulated expression in the *Mecp2*+/- cortex is modulated by dietary vitamin D. Genes whose expression is normalized with vitamin D supplementation are involved in dendritic complexity, synapses, and neuronal projections, suggesting that the rescue of their expression could underpin the rescue of neuronal morphology. Further, motor and anxiety-like behavioral phenotypes in *Mecp2*+/- mice correlate with circulating vitamin D levels, and there is a disruption in the homeostasis of the vitamin D synthesis pathway in *Mecp2*+/- mice. Thus, our data indicate that vitamin D modulates RTT pathology and its supplementation could provide a simple and cost-effective partial therapeutic for RTT.

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Poster

P053. Rett Syndrome II

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Program #/Poster #: P053.02

Topic: A.07. Developmental Disorders

Title: Rapid Phenotypic-based Target Identification, Hit Stratification, and Clinical Translation Using Human iPSC-derived Neurospheroids to Improve Early Identification of Safe and Effective Compounds

Authors: P. NEGRAES, N. SODHI, I. FERNANDES, D. PHAN, ***B. ANSON**, B. VAN HESE, C. TRUJILLO, C. CARROMEU;
Stemonix, Minnesota, MN

Abstract: iPSC-derived neurospheroids offer a promising *in vitro* cellular tool for human-first, early-stage studies to rapidly and accurately screen compound safety and efficacy. Three-dimensional, iPSC-generated models created from clinical populations have been shown to have altered, *in vitro* phenotypes that can be 'rescued' by compounds, followed by target identification and hit stratification from selected reference libraires and combinatorial analytical approaches. Here we generated iPSC-derived neurospheroids from healthy and patient populations, characterized their unique phenotypes, and used them to identify compounds that rescue the disease phenotype without inducing neurotoxicity. Neurospheroids in 384-well plates underwent chronic treatment with a library of 296 well-characterized compounds followed by simultaneous multiplexed functional, structural, and viability evaluation for phenotypic rescue and/or neurotoxicity. Functional assessment employed FLIPR-based readouts of neural activity; over 70 individual endpoints were quantified and used to rank-order rescue while multiplexed, structural, and viability assessments were performed with microscopy-based and ATP-based endpoints to determine potential adverse compound interactions. Example results include 1) compounds that showed toxic responses without any phenotypic rescue; 2) compounds that showed various levels of rescue with evidence of toxicity at higher doses; and 3) 27 compounds that showed good to excellent rescue without any functional, structural, or viability-based toxicity. Compounds eliciting phenotypic recovery spanned several cellular pathways including (but not limited to) the serotonergic, dopaminergic, cholinergic, and GABAergic pathways. Further dissection of cholinergic-based rescue revealed that the primary effect was due to inhibiting the action of acetylcholinesterase. Our results demonstrate a highly specific platform for simultaneously uncovering potential neurotoxicity while identifying the most effective compounds capable of restoring neural function from both healthy and patient-derived material and provide a promising *in vitro* cellular model for early-stage, human-first studies to identify the safest and most effective compounds for pipeline progression and decreased late-stage attrition.

Disclosures: P. Negraes: A. Employment/Salary (full or part-time):; StemoniX. **N. Sodhi:** A. Employment/Salary (full or part-time):; StemoniX. **I. Fernandes:** A. Employment/Salary (full or part-time):; StemoniX. **D. Phan:** A. Employment/Salary (full or part-time):; StemoniX. **B. Anson:** A. Employment/Salary (full or part-time):; StemoniX. **B. Van Hese:** A. Employment/Salary (full or part-time):; StemoniX. **C. Trujillo:** A. Employment/Salary (full or part-time):; StemoniX. **C. Carromeu:** A. Employment/Salary (full or part-time):; StemoniX.

Poster

P053. Rett Syndrome II

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Program #/Poster #: P053.03

Topic: A.07. Developmental Disorders

Support: NIH P50 HD103555 (Cam Core)
NIH P50 HD103555 (Research Project)

Title: Novel unsupervised machine learning approaches for discovering brain state dynamics in human sleep EEG data

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Abstract: Brain activity during sleep is highly dynamic and can be severely altered in patients with neurodevelopmental disorders. Sleep brain state is typically classified using manual polysomnography (PSG) into 5 stages (wake; rapid eye movement, REM; and non-REM 1, 2, 3). This approach presents three major issues: 1) manual PSG is time-consuming and subjective, 2) it has low temporal resolution (typically in 30 seconds segments), and 3) the number and characteristics of sleep stages motivated by normal human sleep might be inappropriate for the abnormal brain states in neurodevelopmental disorders. Here, we use a Hidden Markov Model (HMM) approach to describe brain activity during sleep as rapid (2-sec long) epochs of quasi-stable brain states. We then compare the HMM state dynamics between healthy subjects and Rett syndrome patients and further compare to conventional manual scoring on the same subjects. Five channels (Fp1, F7, C4, O1, T4) electroencephalogram (EEG) was obtained from 22 healthy children and 18 girls with Rett syndrome. Power spectra were filtered to 7 frequency bands: slow delta (0.5-1.5 Hz), fast delta (1.5-4 Hz), slow theta (4-6 Hz), fast theta (6-8 Hz), alpha (8-12 Hz), slow sigma (12~14 Hz), and fast sigma (14~ 16Hz) and used as the model input. A Gaussian HMM was employed to infer a number of discrete states, each of which was modelled as a multivariate normal distribution. Variational Bayes inference was used to estimate the model parameters, including initial states probability, the state statistics, and inter- and intra-state transition probabilities. For the control group, we found that 9 inferred states can well describe the sleep EEG statistics. Some states were highly specific to conventional PSG stages, while others appeared to capture the micro-structure of ambiguous, perhaps transitional stages. The transition matrix captured the trajectories of switching within and between stages, showing prominent transitions from NREM3 to REM. In Rett, the number of inferred states varied between individuals and no states were highly specific to the REM stage, suggesting within and cross-subject REM heterogeneity. Transitions from states highly specific to NREM stages, to states specific to putative wake stages, were frequent compared to controls. A leave-one-out validation procedure showed that the model trained on controls poorly fits Rett data. The proposed HMM framework can help discover short-lived transient brain states, the

heterogeneities in the Rett group, and the difference between the control and Rett during sleep, complementing traditional PSG and providing new windows into brain state pathology in neurodevelopmental disorders.

Disclosures: C. Huang: None. **A. Mahat:** None. **M. Maletic-Savatic:** None. **A. Buckley:** None. **M. McGinley:** None.

Poster

P053. Rett Syndrome II

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P053.04

Topic: A.07. Developmental Disorders

Support: Loulou Foundation

Title: Towards artificial escape of the CDKL5 gene by in vivo DNA methylation editing

Authors: *J. HALMAI[1], C. GONZALEZ[2], F. BUCHANAN[3], J. L. CARTER[5], J. WALDO[3], D. CAMERON[3], P. DENG[4], K. FINK[6];
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Abstract: A disproportionate amount of genes causative for intellectual disability are located on the X chromosome. There are approximately 140 X-linked intellectual disability (XLID) genes with 27% of genes demonstrating a similar or more severe clinical manifestation in females than males. Few treatment options currently exist for these disorders. In somatic cells of female carriers, one X chromosome randomly becomes epigenetically inactivated. This leaves a druggable, silenced wildtype allele behind, which has the potential to rescue phenotypes when the this gene is reactivated. A significant number of X-linked genes escape from the X chromosome inactivation dosage compensation mechanism in female somatic cells. These escape genes are associated with a distinct epigenetic signature. One epigenetic modification that strongly correlates with escape from XCI that we sought to recapitulate in XLID target genes is reduced DNA methylation in promoter regions. In our previous publication we demonstrated artificial escape by editing DNA methylation on the promoter of *CDKL5*, a gene causative for an infantile epilepsy, from the silenced X-chromosomal allele in human neuronal-like cells. We identify that a fusion of the catalytic domain of TET1 to dCas9 targeted to the*CDKL5* promoter using three guide RNAs causes significant reactivation of the inactive allele in combination with

removal of methyl groups from CpG dinucleotides. Strikingly, we demonstrate that co-expression of TET1 and a VP64 transactivator have a synergistic effect on the reactivation of the inactive allele to levels above 60% when compared to the active allele. We further used a multi-omics assessment to determine potential off-targets on the transcriptome and methylome. We find that synergistic delivery of dCas9 effectors is highly selective for the target site. Our findings further elucidate a causal role for reduced DNA methylation associated with escape from X chromosome inactivation. Delivery of the catalytic domain of TET1 fused to dCas9 via adeno-associated virus (AAV) is restricted due to the limited packaging size of the AAV genome. Here, we use dual AAVs for the delivery of trans-spliced dCas9-intein fusion proteins that are then reconstituted in the mouse brain. Optimization of the dual AAVs enables transcriptional reprogramming and targeted DNA demethylation in a mouse model of *Cdkl5* deficiency and demonstrate therapeutic efficacy of our approach. Understanding and remodeling the epigenetics associated with escape from X chromosome inactivation in human neuronal-like cells and *in vivo* has potential for those suffering from X-linked neurodevelopmental disorders.

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Poster

P053. Rett Syndrome II

Location: Virtual Only

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Program #/Poster #: P053.05

Topic: A.07. Developmental Disorders

Support: International Development Research Centre of Canada

Title: The temporoammonic (TA) input to CA1 synapse is hyperexcitable in MeCP2 -/Y mice

Authors: *A. ASGARIHAFSHEJANI, J. PRESSY, M. SERRANILLA, M. WOODIN; Univ. of Toronto, Toronto, ON, Canada

Abstract: Rett syndrome (RTT) is a neurodevelopmental disorder caused by mutations in the X-linked methyl-CpG-binding protein 2 (MeCP2); MeCP2 regulates chromatin organization and global gene transcription. RTT patients demonstrate severe impairments in learning and memory due to alterations in hippocampal network connectivity. In the entorhinal cortex (EC), most neurons in layers II and III have severe synaptic dysfunction. The temporoammonic (TA) pathway provides input directly from layer III of the EC to the distal dendrites of CA1 pyramidal neurons in the stratum lacunosum-moleculare (SLM) of the hippocampus. The TA pathway synapses influence the firing of CA1 pyramidal neurons and modulate the flow of information to the hippocampus. Despite the importance of the TA pathway synaptic input to the CA1 pyramidal neurons for its influence on the firing of CA1 pyramidal neurons, spatial navigation,

learning, and memory, this pathway has not been fully characterized in the context of Rett syndrome. The objective of this study is to electrophysiologically characterize the TA-CA1 and schaffer collateral (SC) -CA1 pathways in a MeCP2-mouse model of RTT to determine the effect of MeCP2 mutations on synaptic and network connectivity. To test this objective, we made whole-cell patch clamp recordings in the hippocampus of Mecp2-/Y mice. The SLM neurons in Mecp2-/Y mice were found to be hyperexcitable showing increased action potential firing rate, with a significantly larger sag potential and post inhibitory rebound potential observed compared to matched wild-types. Additionally, evoked EPSCs of CA1 pyramidal and SLM neurons were significantly larger under TA-CA1 pathway stimulation, with no change in evoked IPSCs compared to wild-types. Moreover, the reversal potential for GABA (EGABA) was measured using a high intracellular chloride concentration (30 mM) and found to be depolarized compared to wild-types controls. Finally, to assess network connectivity we examined hippocampal long-term potentiation induction and paired pulse ratio, which were both found to be unaffected in TA-CA1 and SC-CA1 pathway in the MeCP2-/Y mouse.Taken together, these data demonstrate that CA1 pyramidal neurons have increased intrinsic excitability, revealing a potential mechanism of changes in GABAergic signaling and providing preliminary evidence for a novel mechanism that may contribute to synaptic dysfunction and disease progression.

Disclosures: A. Asgarihafshejani: None. **J. Pressy:** None. **M. Serranilla:** None. **M. Woodin:** None.

Poster

P053. Rett Syndrome II

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Program #/Poster #: P053.06

Topic: A.07. Developmental Disorders

Support: NIH Grant P50HD10353
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Title: Potentiation of mGluR(7) rescues neurophysiological features in a mouse model of Rett Syndrome

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Abstract: Rett syndrome (RTT) is a neurodevelopmental disorder (NDD) primarily caused by mutations in the *Methyl-CpG binding protein 2* (*MECP2*) gene. Currently, there are no well-validated biomarkers in RTT to predict disease prognosis or treatment responses. Previously, we characterized the changes of a variety of neurophysiological features in female R255X *Mecp2* mutation mice and found that specific changes were associated with disease severity, supporting the feasibility of utilizing neurophysiological assessments as non-invasive and translatable biomarkers to predict disease progress and evaluate treatment responses. We have recently found that metabotropic glutamate receptor 7 ($mGlu_7$), a G protein-coupled receptor that regulates presynaptic neurotransmitter release, is reduced in brain tissue from people with RTT and RTT mouse models. Treatment of RTT mice with a compound that acts as a positive allosteric modulator (PAM) of $mGlu_7$ activity (VU0422288) improved phenotypes, suggesting a novel therapeutic approach for RTT. We investigated whether $mGlu_7$ PAM treatment could alter neurophysiological assessments in RTT animals to evaluate their potential of serving as pharmacodynamic or early treatment response biomarkers. Our results showed that acute dosing of VU0422288 (30mg/kg, i.p.) in wildtype female mice did not have any effect on auditory evoked potential (AEP) form or peak amplitudes. However, acute injection of VU0422288, but not vehicle, improved the AEP waveform and increased AEP N1 and P1-N1 peak amplitudes in female $Mecp2^{Null/+}$ mice. Additionally, both auditory evoked power spectrum and inter-trial phase coherence were also improved in RTT animal by VU0422288 administration. These findings suggest that potentiation of $mGlu_7$ functionally improved neural circuit synchrony to auditory stimuli in RTT animals, and that this acute response could represent a pharmacodynamic biomarker useful for dose finding and potentially as an early treatment response biomarker.

Disclosures: H. Dong: None. **K. Weiss:** None. **K. Baugh:** None. **C.M. Niswender:** None. **J.L. Neul:** None.

Poster

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Program #/Poster #: P053.07

Topic: A.07. Developmental Disorders

Support: R01NS120746
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Title: Rett Syndrome mice have aberrant astrocyte gene expression and morphological maturation during CNS refinement

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Abstract: Rett Syndrome (RTT) is an X-linked neurodevelopmental disorder caused by mutations in the MECP2 gene. The MeCP2 protein is a critical transcriptional regulator in the CNS responsible for neurotypical function and maturation. Persons with RTT experience symptom onset after a period of typical development between 6 to 18 months of age, causing regressions in motor abilities, development of breathing abnormalities, and intellectual impairment. $Mecp2^{-/y}$ mouse models similarly recapitulate symptoms and onset, providing a basis for understanding cellular contribution to disease progression. $Mecp2^{-/y}$ mouse studies have specifically demonstrated that astrocytes contribute to RTT pathogenesis, but the exact mechanisms are not well understood. Astrocytes undergo postnatal maturation alongside neurons (~P14 - P30), indicated by changes in cellular function, and development of a complex morphological phenotype. Interestingly, $Mecp2^{-/y}$ mice first display symptom phenotypes during this period of astrocyte refinement. Using cell-specific isolation of cortical astrocytes, we used next-generation RNA-Sequencing to evaluate gene expression in WT and $Mecp2^{-/y}$ male mice from early postnatal development through adulthood (P7, P14, P28, P45, and P60, n = 3-5 per genotype x age). We found the highest number of significant ($p < 0.05$) differentially expressed genes (DEGs) during the period of murine astrocyte morphogenesis (P14 - P28) in WT animals. Comparison of WT vs. $Mecp2^{-/y}$ cortical astrocytes at P14 and P28 time points revealed 33 DEGs and 7,251 significant DEGs between groups, respectively. Gene Ontology pathway analysis was only possible for P28 time points given the low DEGs at P14, where we found that WT astrocytes have pathway enrichment in cellular morphology and maturation, while these pathways are disrupted in $Mecp2^{-/y}$ astrocytes. Evaluating astrocyte morphology using an AAV construct for visualization, we determined that $Mecp2^{-/y}$ animals have deficits in astrocyte morphogenesis at the end of this developmental period at P30, but not prior to it at P14. These results suggest that developmental disruption of astrocyte morphogenesis and gene pathways of maturation in RTT may have functional relevance that further implicate astrocytes as disease contributors.

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Poster

P169. Glia-Neuron Interactions in Physiology I

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P169.01

Topic: B.11. Glial Mechanisms

Support: EU Grant ITN 764860

Title: The neuropeptide Galanin exerts inhibitory effects on cortical spreading depolarization via the Galanin receptor 2

Authors: F. GIMENO-FERRER[1], A. EITNER[2], R. BAUER[3], A. LEHMENKUHLER[4], H.-G. SCHAIBLE[1], ***F. RICHTER**[1];
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Abstract: The neuropeptide Galanin reduces neuronal excitability in rat cerebral cortex, increases the threshold for elicitation and interferes with amplitudes and propagation velocities of cortical spreading depolarization (CSD). Here we tested, whether the Galanin receptor 2 (GalR2) could be the reason for these effects and checked its localization. In spontaneously breathing anesthetized adult rats (sodium thiopentone, 100 mg/kg, i.p.) the electrocorticogram was recorded with arrays of glass microelectrodes in two areas (treated and untreated) that were separated by a wall made from dental acrylic. Recordings were performed in both areas at cortical depths of 400 and 1200 µm. CSD was induced by KCl microinjection. CSD-related direct current (DC) potential shifts, changes in extracellular potassium concentration and in regional cerebral blood flow were continuously monitored. In group 1 we applied the GalR2 antagonist M871 at a concentration of 3 nM for 2 h as pretreatment, followed by 2 h application of Galanin at 0.1 µM. In group 2, the $GABA_A$ receptor antagonist Bicuculline at 1µM was applied for 1 h alone followed by Galanin at 1 µM for 3 h. In group 3, a mixture of both with same concentrations was applied for 3 h. Native rat brain slices were stained for the GalR2 and for parvalbumin interneurons. The GalR2 antagonist alone reduced the CSD amplitudes in treated brain area for 30 to 60 min, and then amplitudes returned to normal. A subsequent application of Galanin did not further influence CSD. The pretreatment with Bicuculline caused some spontaneous CSD starting in the treated area and propagating across the whole cortex. A subsequent application of Galanin did not affect CSD. In animals with coapplication of Bicuculline and Galanin no spontaneous CSD occurred and CSD were not influenced either suggesting that a neuronal network is involved in Galanin functions. The precise localization of GalR2 is under investigation. We conclude that Galanin controls cortical neuronal activity via the GalR2, since its blockade prevented all effects on CSD. The experiments with Bicuculline indicate that a GABAergic mechanism may be involved in the diminishing and slowing of CSD by Galanin.

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Poster

P169. Glia-Neuron Interactions in Physiology I

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Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P169.02

Topic: B.11. Glial Mechanisms

Support: NIH Grant U19NS104653
NIH Grant R43OD024879
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Title: Molecular and anatomical dissection of a neural circuit for futility-induced passivity

Authors: *A. B. CHEN[1,2], M. ZHU[3], S. NARAYAN[1], M. PETKOVA[3], M. RUBINOV[5], J. S. MARVIN[1], B. S. KHAKH[6], L. L. LOOGER[1], J. W. LICHTMAN[4], F. ENGERT[3], M. B. AHRENS[1];
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Abstract: To survive, animals must generate appropriate behaviors in response to dynamic environmental stimuli and internal goals. How the brain evaluates external and internal evidence and changes behavioral strategies when necessary is an important but poorly understood problem in neuroscience. We study behavioral strategy switch in the case of futility-induced passivity in the larval zebrafish. Previous work demonstrated that larval zebrafish stop swimming when swims are ineffective and identified a hindbrain circuit consisting of both neurons and glia that integrates evidence of failure and inhibits swims. Here, further characterize this neural-glial circuit with a goal of understanding the molecular, cellular, and systems mechanisms through which it operates. To investigate the molecular mechanisms through which radial astrocytes integrate the previously characterized norepinephrinergic failure signal and communicate with downstream neurons, we employed simultaneous light-sheet imaging of intracellular astrocytic calcium levels and other astrocytic intercellular and extracellular signals, such as potassium concentration and ATP release. We identified correlates of futile swims, passivity, and recovery to activity in the ATP signal. To investigate the anatomical features of the futility-induced passivity circuit, we reconstructed noradrenergic hindbrain cells that signal futility, radial astrocytes that integrate the futility signal, and hindbrain GABAergic interneurons that inhibit swimming. By mapping connections between these medullary GABAergic neurons and their downstream partners, we uncovered anatomical evidence for how swimming is inhibited by futility.

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Poster

P169. Glia-Neuron Interactions in Physiology I

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Program #/Poster #: P169.03

Topic: B.11. Glial Mechanisms

Support: K08NS102526 NIH/NINDS (CWH)
2020 Doris Duke Charitable Foundation Clinical Scientist Career Development Award (CWH)

Title: An in vitro model of human neural development indicates human specific astrocyte effects on neuronal network maturation

Authors: S. LIU[1], A. TAGA[2], A. JOHNS[1], N. J. MARAGAKIS[3], *C. W. HABELA[2]; [2]Neurol., [1]Johns Hopkins Univ., Baltimore, MD; [3]Dept Neurol, Johns Hopkins Univ. Dept. of Neurol. and Neurosurg., Baltimore, MD

Abstract: Neurodevelopmental disorders such as epilepsy and intellectual disability result from disruption of cortical neural development. This can occur due to changes in neural cell proliferation, migration, synaptic integration and network organization and may be a result of deficits in either neurons or glial cells or in the interactions between these cells. The study of cortical neurodevelopmental disorders with genetic causes has been facilitated by the development of human induced pluripotent stem cells (iPSC) which allow patient derived cells to be studied in the context of human genetic backgrounds. While there are numerous well characterized human cortical neuronal differentiation protocols from iPSCs, models in which both neurons and astrocytes are generated from a single progenitor are less well characterized and many studies of neuronal development rely on rodent astrocytes to facilitate synaptic development. To more closely approximate early neural development, we developed a protocol to generate astrocyte progenitors from forebrain neural progenitor cells previously validated to generate forebrain cortical neurons. When these astrocyte progenitor cells were matured until day in vitro (DIV) 95-100 and characterized by fluorescent immunocytochemistry, greater than 75% expressed the astrocytic markers GFAP and S100β indicating an astrocytic fate. Greater than 95% of cells expressed the cortical region-specific transcription factor OTX2 while only a small minority of the cells expressed the spinal specific transcription factor, HOXB4, indicating a cortical astrocyte fate. We subsequently cultured DIV 37 human cortical neurons (hCN) with either DIV 90 human cortical astrocytes (hCA), cortical rat astrocytes or no astrocytes on multielectrode array plates and recorded extracellular field potentials of the cocultures for 1 to 6

months. All co-cultures including hCA demonstrated robust spike activity within three days of plating, burst activity within 2 weeks, and synchronized bursts across the network between 1 and 2 months after plating. Interestingly, although hCN cultured with hCA all developed mature network burst patterns, this maturation occurred over a more protracted time course when compared to co-cultures including rat astrocytes. This is consistent with the slower rate of neural development in humans compared to rats and emphasizes important differences between fully human cultures and hybrid human-animal models. It also confirms the importance of astrocytes in neuronal development and network activity and supports the use of both astrocytes and neurons from patient iPSCs when studying neurodevelopmental disorders.

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Poster

P169. Glia-Neuron Interactions in Physiology I

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Program #/Poster #: P169.04

Topic: B.11. Glial Mechanisms

Support: NIH Grant KO1MH105040-01
NIH Grant R01MH052804-28
BBRF Young Investigator Grant

Title: Compartment-specific neurexin nanodomains orchestrate tripartite synapse assembly

Authors: *J. TROTTER[1], Z. DARGAEI[2], A. SCLIP[1], S. ESSAYAN-PEREZ[1], K. LIAKATH-ALI[1], K. RAJU[1], A. NABET[1], X. LIU[3], M. WÖHR[4], T. C. SUDHOF[1];
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Abstract: At tripartite synapses, astrocytes enmesh synaptic contacts, but how astrocytes contribute to the formation, maturation and plasticity of synapses remains elusive. Here we show that both astrocytes and neurons abundantly express neurexin-1, a presynaptic adhesion molecule that controls synaptic properties. Using super-resolution imaging, we demonstrate that presynaptic neuronal and astrocytic neurexin-1 form discrete nanoclusters at excitatory synapses. We find that distinct patterns of heparan sulfate modification and alternative splicing confer onto astrocytic and neuronal neurexin-1 different ligand specificities, thereby enabling compartment-specific signaling by neurexin-1. At hippocampal Schaffer-collateral synapses, deletion of neurexin-1 from either astrocytes or neurons did not alter synapse numbers, but differentially impaired synapse function. Neuronal neurexin-1 was essential for NMDA-receptor-mediated synaptic responses, whereas astrocytic neurexin-1 was required for maturation of silent synapses,

AMPA-receptor recruitment, and long-term potentiation. Thus, astrocytes and neurons surprisingly use the same synaptic adhesion molecule to control distinct synapse properties.

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Poster

P169. Glia-Neuron Interactions in Physiology I

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Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P169.05

Topic: B.11. Glial Mechanisms

Support: NIH Grant R01EY027826
NIH Grant R01EY021796

Title: A neuron-glia signal strengthens inhibitory synaptic transmission to attain fly dim-light vision

Authors: *P. GUO, Z. LUAN, H.-S. LI;
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Abstract: Inhibitory neurotransmission participates in nearly every neural circuit and plays fundamental role in shaping and balancing neuronal signaling. Fast synaptic inhibition in the central nervous system is primarily mediated by the Cl^- influx through ligand-gated chloride channels. Therefore, the activation of these postsynaptic receptors has drawn most attention in previous regulation mechanism studies of inhibitory synaptic transmission. In *Drosophila*, visual information is relayed from photoreceptors to large monopolar cells (LMCs) through histamine-gated chloride channels. Here we report that this inhibitory synaptic transmission is strengthened by a glutamatergic neuron-glia signaling process to attain vision in dim conditions. Fly outer photoreceptors innervate LMCs in the first visual neuropil lamina, which also contains terminals of multiple types of interneurons. The epithelial glia (EG) spans the entire thickness of the lamina, extends processes into spaces between neurons and envelops synapses. We identified a glutamate-gated chloride channel (GluCl) that is localized on an EG membrane structure gnarl. Loss of glial GluCl significantly reduced the light-sensitivity of LMCs but not that of photoreceptors, and impaired the fly response to moving gratings in an optomotor assay when the background illuminance is below 100 lux. The visual defect was reversed by expressing a Cl^--extruding K^+-Cl^- cotransporter KCCB in glia, indicating that the activation of GluCl may release Cl^- from glia to increase the driving force for Cl^- flux into LMCs, thereby reinforcing the visual synapse in dim conditions. *in vivo* imaging using glutamate sensor and membrane potential sensor showed a laminar extracellular glutamate level ($[Glu]_o$) increase in dark that depends on

the depolarization of T1 interneuron. In the lamina, an electrogenic glutamate transporter EAAT1 is exclusively expressed in T1. Knockdown of EAAT1 in T1 cells completely abolished the light triggered $[Glu]_o$ change, and led to similar visual defects observed in GluCl knockdown flies, suggesting that the opening the GluCl-gated glial Cl^- pool is triggered by the EAAT1-mediated glutamate release from T1 cells. This work has demonstrated that the inhibitory neurotransmission can be rapidly modulated by glia under the control of a third neuron, which may represent a general glial regulation on the gain of inhibitory synapses.

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Poster

P169. Glia-Neuron Interactions in Physiology I

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Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P169.06

Topic: B.11. Glial Mechanisms

Support: Brown University Undergraduate Teaching and Research Award (UTRA)

Title: Ectopic action potential activation in regular-spiking neurons in the neocortex

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Abstract: Objectives: This study aims to determine whether a form of backpropagating action potentials known as ectopic firing occurs in regular-spiking (RS) cells under non-pathological conditions. Prior work suggests that at least two types of inhibitory interneuron fire action potentials that originate in the axon (Elgueta et al., 2015; Imbrosci et al., 2015). There is a large body of evidence indicating that RS cells fire ectopic action potentials in seizure models, but we do not yet know whether they are capable of ectopic firing under non-pathological conditions. Here, we show preliminary data suggesting that ectopic firing occurs in mouse neocortical pyramidal cells.
Methods: We recorded from RS cells in layer 2/3 of the mouse orbitofrontal cortex utilizing the whole-cell patch clamp technique *in vitro* and attempted to induce ectopic firing using multiple protocols. To elicit ectopics, we ran 75, 60, 40, and 30 Hz protocols, as well as a protocol in which 600 ms current steps were incremented by +5 pA every 2 seconds. To quantify ectopic firing, we analyzed the number of evoked AP's that were required before the first ectopic, and determined how many ectopic APs were present in the most active sweep. An ectopic AP was defined as one that rose steeply from the baseline membrane potential and occurred greater than 3 milliseconds after the last evoked AP of that sweep.
Results: Among six RS neurons, 83% (n=5) fired ectopic APs. On average, it took 431 ± 455 APs before the arrival of the first ectopic. The most active run for cells one, three, and five

occurred during the 60 Hz protocol, while cell two's was at 75 Hz and cell four's was at 30 Hz. The average number of ectopics in the most active run was 8. Two of the five cells had 5 or less ectopics in their most active runs, while one cell had between 5 and 10. The other two cells had 20 or more ectopics during their most active runs. 100% of the cells had their first ectopic within 82 seconds of the first evoked action potential. The amplitude of the ectopics ranged from high amplitude (greater than the height of the evoked APs in that cell), medium amplitude (slightly lower than the evoked AP), and low amplitude (less than one-fourth of the evoked AP). **Conclusion:** Our data suggest that under non-pathological conditions, RS neurons are capable of firing ectopic APs after being sufficiently stimulated. Future work will determine the percentage of neocortical RS cells that are capable of ectopic firing, and determine the mechanism of ectopic firing.

Disclosures: S.R. Doelfel: None. **B.W. Connors:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; R01 MH115049, NIH; RO1 NS100016, NIH; R01 NS104428-01 NIH. **B.B. Theyel:** 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.; K08NS118114-02.

Poster

P169. Glia-Neuron Interactions in Physiology I

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P169.07

Topic: B.11. Glial Mechanisms

Support: NSERC RGPIN-2020-03971

Title: Evidence for an astrocytic role in somatosensory adaptation

Authors: *M. MACNAB, L. K. BEKAR;
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Abstract: Somatosensory cortical adaptation is the frequency-dependent inhibition of firing in response to background stimulation. At high frequency, this is largely a result of synaptic vesicle depletion. However, at lower frequencies, multiple systems may be at play. As part of the tripartite synapse, astrocytes interact closely with neurons and regulate many factors that influence firing. By regulating $[K^+]_e$, astrocytes can affect the membrane potential and thus neuronal excitability. Various gliotransmitters are also known to be involved in modulating synaptic release (glutamate, adenosine, endocannabinoids) and recruitment of interneuron-mediated inhibition (ATP, glutamate). As astrocytes are ideally positioned to respond to synaptic

activity, express a multitude of receptors, and release gliotransmitters to affect surrounding cells, we ***hypothesize*** that astrocytes play a role in cortical sensory adaptation. Live brain slices were stimulated in cortical layer IV with extracellular field recordings performed in layer II. Ten-pulse stimulation trains (25, 20 and 10 Hz) were used to assess pharmacological and technical manipulations on field EPSP adaptation. Initial patch electrode dialysis of astrocytes with the calcium chelator BAPTA demonstrates that astrocyte calcium signaling plays a prominent role. Lowering of extracellular K^+ from 3 to 1 mM in the artificial cerebral spinal fluid (aCSF) perfusing the slice to help reduce build-up in response to the stimulation train also reduced adaptation, supporting a role for astrocyte-mediated K^+ regulation. Subsequent assessment of pharmacological blockade of $GABA_A$, $GABA_B$, NMDA, P2Y1, and CB1 receptors all showed modest effects on adaptation. Surprisingly, mGluR5 and A1/A2a adenosine receptor blockade had no effect. The effects of astrocyte syncytium dialysis with BAPTA and perfusion with low K^+ aCSF demonstrate that astrocytes play a significant role in somatosensory adaptation. Pharmacological manipulation suggests that astrocytes may use ATP through P2Y1 receptors to recruit interneuron-mediated inhibition and that endocannabinoids from neurons and/or astrocytes also play a role.

Disclosures: M. Macnab: None. **L.K. Bekar:** None.

Poster

P169. Glia-Neuron Interactions in Physiology I

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P169.08

Topic: B.11. Glial Mechanisms

Support: NIH Grant R01NS120746

Title: Examining BDNF/Astrocytic TrkB.T1 signaling as a mechanism underlying perisynaptic astrocyte process recruitment

Authors: *B. TORRES, L. M. HOLT, J. BROWNING, M. L. OLSEN;
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Abstract: Perisynaptic astrocyte processes (PAPs) are fine terminal structures located on the distal processes of astrocytes. These PAPs contain numerous neurotransmitter receptors, ion channels, cell-adhesion molecules, and the ability to release synaptogenic molecules, allowing astrocytes to contribute to synapse homeostasis, development, and stabilization. Despite decades of research indicating astrocytes enwrap or contact synaptic elements, with increased coverage of mature glutamatergic synapses, there is little known regarding the molecular signals that recruit PAPs to synapses or induce structural PAP plasticity in response to neuronal signaling. Brain derived neurotrophic factor (BDNF) has been highly implicated as a molecule underlying both

synapse development and plasticity in neurons. In developing or highly active synapses, BDNF is released from pre- or post-synaptic elements and binds to its receptor TrkB to mediate synapse maturation. Interestingly, our recent published work demonstrates that astrocytes express high levels of TrkB. We identified that the truncated TrkB receptor, TrkB.T1, is the predominant TrkB receptor identified in cortex and is predominantly expressed in astrocytes relative to other CNS cell populations. Direct genetic manipulation of TrkB.T1 globally and in astrocytes alone is sufficient to reduce astrocyte morphological maturation both *in vitro* and *in vivo,* alters mature astrocyte gene expression *in vivo,* and reduces excitatory synapse formation *in vitro.* Our preliminary data indicate that TrkB.T1 knockout (KO) astrocytes fail to enwrap glutamatergic synapses *in vitro*. Ongoing studies using *in vitro* astrocyte neuron co-cultures and experience dependent plasticity in the mouse whisker barrel cortex *in vivo* are aimed at examining BDNF/TrkB.T1 signaling as a mechanism underlying PAP arrival and plasticity at developing synapses. Alterations in synaptic function and development have been implicated in multiple neurodevelopmental disorders. This work will advance the understanding of the role of astrocytes in synaptic development and offer new therapeutic targets where synapse development is implicated.

Disclosures: B. Torres: None. **L.M. Holt:** None. **J. Browning:** None. **M.L. Olsen:** None.

Poster

P169. Glia-Neuron Interactions in Physiology I

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P169.09

Topic: B.11. Glial Mechanisms

Title: Purinergic signaling in glia is necessary for foraging and food consumption in drosophila.

Authors: *J.-F. DE BACKER, T. KARGES, J. PAPS, I. C. GRUNWALD KADOW;
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Abstract: Like most animals, hungry flies have to start to seek for food supply. In order to find a food source, flies first use olfactory and visual cues. If successful, they then assess the type and the quality of the food and eventually start feeding. As foraging, feeding and digestion are also energy-costly activities, these behaviors must be repressed when the animal reaches satiation. This information is given by post-ingestive feedback over the quantity and the nutritive quality of the ingested food. All along an animal's life, internal resources such as energy stores must therefore be evaluated and allocated to the appropriate system according to the current metabolic state. In the central nervous system, glial cells seems to be good candidates to play that role. They are known to provide metabolic support to neurons but can also modulate their excitability as well as synaptic transmission. A recently increasing amount of data shows that glia activity can have a direct impact on various types of behavior in both vertebrates and invertebrates. Here,

we hypothesize that glial cells could sense the metabolic state of the animal and then promote and/or repress the decision of engaging food-seeking and feeding behavior. We are addressing this hypothesis in food-deprived *Drosophila* using food-odor tracking on a spherical treadmill and a single-fly feeding behavior assay. We observed that the expression of the adenosine receptor (AdoR) in glial cells is necessary in both cases. Interestingly, the role of AdoR seems to be different according to the cell types composing *Drosophila* glia population. Adenosine is often presented as a stress-signaling molecule and has for example been shown in flies to promote the allocation of internal energy stores to immune cells during an infection. Adenosine is also the product of ATP dephosphrylation and could then potentially be used by cells to sense energy level and/or consumption. As AdoR is an excitatory GPCR, we activated the different glia sub-types using optogenetics. Again, the activation of different cell-types inhibited or enhanced food-odor tracking behavior in starved flies. Together, our data suggest that different populations of glial cells could possibly sense the metabolic state of the fly and orient its behavior toward food-seeking or feeding.

Disclosures: J. De Backer: None. **T. Karges:** None. **J. Paps:** None. **I.C. Grunwald Kadow:** None.

Poster

P169. Glia-Neuron Interactions in Physiology I

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P169.10

Topic: B.11. Glial Mechanisms

Title: Mechanism of NMDA Receptor Potentiation by Lactate

Authors: *H. FIUMELLI[1], G. HERRERA-LOPÉZ[1], F. LEMTIRI-CHLIEH[2], N. CARRANO[3,4], F. GARDONI[3], M. DI LUCA[3], P. J. MAGISTRETTI[1];
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Abstract: Astrocyte-derived lactate is known to have multiple effects on neuronal cells, fueling high-energy demands as a metabolic substrate and acting as a trophic signaling molecule to promote synaptic plasticity and memory consolidation. Recent findings have shown that lactate regulates NMDA receptor activity through a NADH-dependent pathway and modulates gene expression related to synaptic plasticity and neuroprotection. To further elucidate the mechanism of NMDA receptor potentiation by lactate, we used patch clamp recordings in primary cultures of cortical neurons, and found that lactate rapidly enhanced both the amplitude and the inactivation time-constant of NMDA receptor currents (I_{NMDAR}) evoked by brief applications of glutamate and glycine. These effects were not observed by agonists of HCAR1/gpr81 receptors while the lactate-mediated increase in I_{NMDAR} amplitude was prevented by monocarboxylate

transporters and lactate dehydrogenase inhibitors. Moreover, intracellular infusion of specific CaMKII peptide inhibitors also abolished the potentiation of peak I_{NMDAR} responses by lactate, indicating a mechanism requiring entry of lactate into neurons, its oxidation to pyruvate, and involvement of CaMKII. Furthermore, interfering with the binding between CaMKII and GluN2B in a pharmaco-genetic model consisting of CaMKIIα-expressing HEK293 cells transiently transfected with functional NMDA receptors containing mutant GluN2B subunits prevented the potentiation of I_{NMDAR} responses by lactate. Interestingly, proximity ligation assays in cultured neurons between GluN2B and the postsynaptic density associated scaffolding protein PSD-95 revealed that lactate induced GluN2B accumulation in dendritic spines, an effect that was prevented by CaMKII inhibitors. Taken together, these observations suggest that lactate uptake stimulates synaptic plasticity by increasing the distribution of NMDA receptors in spines by a mechanism promoting the interaction between CaMKII and GluN2b subunits.

Disclosures: H. Fiumelli: None. **G. Herrera-Lopéz:** None. **F. Lemtiri-Chlieh:** None. **N. Carrano:** None. **F. Gardoni:** None. **M. Di Luca:** None. **P.J. Magistretti:** None.

Poster

P205. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models IV

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P205.01

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

Support: NIH RF1 AG060057

Title: Global Complement C3 depletion protects against synapse loss, long-term potentiation impairment and alleviates cognitive decline in aged mice

Authors: *A. BATISTA[1,2], M. SCHROEDER[4], K. KHAN[4], S. LI[1,2], J. PRESUMEY[5,3], E. YALCIN[5,3], M. CARROLL[5,3], C. A. LEMERE[6,7];
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Abstract: The complement cascade modulates the innate immune system, which leads to the elimination of pathogens and dead or dying cells, as well as contributing to the extent and limit of the inflammatory immune response. Complement C3 is part of the innate immune system and participates in synapse elimination during normal brain development to refine neuronal networks. Further, this molecule may be detrimental in several neurodegenerative diseases, including Alzheimer's disease (AD). C3 levels in the brain are elevated during aging and AD.

Our group previously showed that lifelong C3-deficiency protected against hippocampal synapse loss in aged WT and APP/PS1dE9 mice, correlating with improved performance on cognitive tests. Recently, we developed an inducible conditional C3 knockout mouse model (C3iKO) by crossing C3 floxed ($C3^{fl/fl}$) mice with Rosa26-Cre-ERT2+/- mice on a C57BL/6J background. Our goal was to investigate whether C3 lowering in adulthood, after normal brain development, would still protect hippocampal function in these mice. First, 4-5-month-old male and female C3iKO mice were i.p. injected with tamoxifen (TAM) or corn oil (CO; control) daily for 5 days. Tamoxifen treatment of C3iKO mice led to a sustained ~95% lowering of serum C3 levels. Behavioral testing for hippocampal-dependent spatial memory, object memory, and object location were performed when TAM-treated and CO-treated mice reached 16-17 months of age. C3iKO+TAM mice performed significantly better than C3iKO+CO-treated mice in these behavioral tasks, indicating that C3 lowering after brain development protected mice from age-related cognitive decline. In another study, mice were treated with TAM or CO at 3-4 months of age and electrophysiological recording of long-term potentiation (LTP) was conducted 3 months later in hippocampal slices incubated with neurotoxic $(A\beta_{1\text{-}40}\ S26C)_2$. Remarkably, C3 lowering protected hippocampal synapses from $(A\beta_{1\text{-}40}\ S26C)_2$-mediated LTP impairment. In conclusion, global C3 lowering in C3iKO young adult mice protected against hippocampal dysfunction many months later, suggesting that targeting C3 may be an effective therapeutic strategy for AD.

Disclosures: A. Batista: None. **M. Schroeder:** None. **K. Khan:** None. **S. Li:** None. **J. Presumey:** None. **E. Yalcin:** None. **M. Carroll:** None. **C.A. Lemere:** F. Consulting Fees (e.g., advisory boards); Probiodrug AG, Cognition Therapeutics, DIAN TU-Therapeutic Evaluation Panel, Alz Assoc MSAC, Amgen, Alzheon, Biogen.

Poster

P205. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models IV

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P205.02

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

Support: NIH RF1 AG058081
NIH RF1 AG056976
University of Minnesota College of Pharmacy

Title: Impact of TREM2 in a Transgenic Mouse Model of Cerebral Amyloid Angiopathy

Authors: *R. ZHONG, A. GRAM, L. LI;
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Abstract: Cerebral amyloid angiopathy (CAA) is characterized by cerebral vascular deposition of pathological amyloid-beta protein (Aβ), triggering cerebrovascular inflammation,

hemorrhages, and cognitive impairment. CAA plays an important role in the multimorbidity of aging brains including Alzheimer's disease (AD), but it is less understood compared to the other more heavily studied Aβ pathology in AD, Aβ plaques. Currently, there is no approved therapeutic agent to specifically treat CAA. Triggering receptor expressed on myeloid cells 2 (TREM2), a key player in innate immune response, is exclusively expressed on microglia in the brain and mediates Aβ clearance by microglia and subsequently relieves neuroinflammation, among other neuroprotective effects. Recent studies on TREM2 function using TREM2 knockout (KO) in transgenic (Tg) AD mice have shown that TREM2 modulates parenchymal Aβ deposition in the brain, although the direction of the effect depends on the specific Tg background and the timing/stage of the disease development. However, the impact of TREM2 on CAA had not been investigated. In this study, we crossed TREM2 KO mice with APPswDI mice (C57BL/6-Tg(Thy1-APPSwDutIowa)BWevn/Mmjax), a model of CAA. We found that TREM2 deficiency markedly increased overall Aβ load in APPswDI/TREM2KO mice compared to APPswDI/TREM2WT or APPswDI/TREM2Het in cortex, hippocampus, and thalamus. Intriguingly, TREM2 deletion led to a dramatic decrease in CAA in vasculature-enriched regions, such as thalamus, in APPswDI mice. Moreover, overall microglial staining (IBA1) and CAA-associated microglia were significantly reduced in APPswDI/TREM2KO mice. Additional experiments are underway to explore the molecular mechanism underlying the changes in CAA and Aβ plaques upon TREM2 deletion. The results provide novel insights into the differential role of TREM2 in the regulation of parenchymal versus vascular deposition of Aβ in the brain.

Disclosures: R. Zhong: None. **A. Gram:** None. **L. Li:** None.

Poster

P205. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models IV

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P205.03

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

Support: NIA Grant R01AG062840
the Joseph H. Stahlberg Foundation

Title: Delayed treatment with a brain-penetrating biologic TNF-α inhibitor in the APP/PS1 mouse model of human amyloidosis

Authors: *W. OU[1], Y. OHNO[2], M. CHOI[3], J. SUN[1], J. YANG[2], T. ABDULLAH[1], R. MURPHY[1], C. ROULES[1], M. CHOI[4], D. H. CRIBBS[5], R. SUMBRIA[1,5];
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Abstract: Tumor necrosis factor-α (TNF-α) plays a vital role in Alzheimer's Disease (AD) pathology and, TNF-α inhibitors (TNFIs), including Etanercept, modulate AD hallmark pathology (Aβ and tau pathology). We engineered a blood-brain barrier (BBB)-penetrating TNFI by fusing the type-II human TNF-α receptor (TNFR) to a transferrin receptor antibody (TfRMAb) that ferries TNFR into the brain non-invasively via receptor-mediated transcytosis. We previously showed that the BBB-penetrating TfRMAb-TNFR is protective when administered to 6-month-old APP/PS1 transgenic mice, however, the effects of delayed TfRMAb-TNFR treatment are not known. Herein, 10.7-month-old male APP/PS1 mice were injected intraperitoneally with saline (n=16), TfRMAb-TNFR (3 mg/kg; n=9), Etanercept (1.5 mg/kg; equimolar dose of TNFR; n=9) or TfRMAb (2.25 mg/kg; equimolar dose of TfRMAb; n=5) three days per week for 10 weeks. Blood was collected at 10 weeks for a complete blood count and white blood cell (WBC) differential. Spatial recognition memory was assessed using the modified Y-maze. Total brain Aβ peptide load was measured by 6E10 immunostaining and insoluble brain Aβ(1-42) was measured by ELISA. Markers of BBB function and amyloid precursor protein were measured by Western blotting in whole tissue homogenates. Biologic TNFIs did not alter hematologic parameters, however, TfRMAb decreased hematocrit ($p<0.0001$) and increased WBC ($p<0.01$) compared to saline-treated mice. Both TfRMAb-TNFR ($p<0.0001$) and Etanercept ($p<0.001$) significantly reduced brain insoluble Aβ(1-42); however, only TfRMAb-TNFR reduced both cortical and hippocampal 6E10-positive Aβ area ($p<0.05$) and number ($p<0.05$) in the APP/PS1 mice. The 6E10-positive Aβ area ($p<0.05$) and number ($p<0.05$) were significantly higher in the Etanercept-treated mice compared to the TfRMAb-TNFR-treated APP/PS1 mice. TfRMAb-TNFR-treated mice spent more time ($p<0.01$) in the novel arm and had reduced latency ($p<0.05$) to enter the novel arm compared to the APP/PS1 saline-treated mice. Etanercept treatment did not improve spatial memory of the APP/PS1 mice. Additionally, TfRMAb-TNFR-treated-, but not Etanercept-treated-mice, had higher BBB-tight junction protein expression ($p<0.05$) compared to saline-treated mice. Overall, delayed treatment with either Etanercept or TfRMAb-TNFR (BBB-penetrating TNFI) reduced insoluble brain Aβ(1-42), however, TfRMAb-TNFR resulted in better therapeutic indices and also reduced 6E10-positive total Aβ peptide load, increased BBB-tight junction protein levels, and improved spatial memory in the APP/PS1 mouse model of human amyloidosis.

Disclosures: W. Ou: None. **Y. Ohno:** None. **M. Choi:** None. **J. Sun:** None. **J. Yang:** None. **T. Abdullah:** None. **R. Murphy:** None. **C. Roules:** None. **M. Choi:** None. **D.H. Cribbs:** None. **R. Sumbria:** None.

Poster

P205. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models IV

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P205.04

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

Support: NIH/NIA RF1-AG055524
NIA 2020-2021 MIND Training Grant

Title: Investigating the role of cytotoxic t cells in alzheimer's disease pathogenesis

Authors: *J. SANCHEZ[1], J. BECK[1], L. MCINTYRE[2], S. KIANI SHABESTARI[2], M. INLAY[1], C. WALSH[1], H. DAVTYAN[1], M. BLURTON-JONES[1];
[1]Univ. of California Irvine, Irvine, CA; [2]Univ. of California, Irvine, Irvine, CA

Abstract: The innate immune system have been strongly implicated in Alzheimer's disease (AD) pathogenesis. In contrast the role of adaptive immunity remains poorly understood. My studies elucidate the specific role of T cell infiltration into the AD brain parenchyma. I have utilized both immune-deficient and immune-intact AD transgenic mouse models. I performed transplantation of either bone marrow hematopoietic stem cells (HSCs) or adoptive transfer of donor splenic T cells into our immune-deficient AD mouse model and wild-type controls. I found a significant increase in Helper T cells, and an even greater increase in memory Killer T cells within RAG2ɣc-/-5xfAD brain parenchyma. Interestingly, T cells were sometimes observed adjacent to plaque-associated microglia in the RAG2ɣc-/-5xfAD brain, suggesting that activated microglia may play a role in T cell recruitment and/or antigen presentation to T cells. I next sought to determine whether a similar effect can be observed within immune intact AD mice. I generated, aged, and examined four genotypes of immune intact mice: wild-type, 5xfAD, PS19 tau mice, and bigenic Tau-5x mice. I confirmed that beta-amyloid induces infiltration of CD8 T cells and that the combination of amyloid and tau pathology induces the most robust infiltration of CD8 T cells. Results also showed the subtypes of CD8 T cells that infiltrate the brains of AD mice are memory T cells, demonstrating that they respond to only a few highly specific brain-derived antigens. I next performed stereological quantification of neuronal numbers within these mice brains and compared these results to flow cytometry quantification of CD8 T cells. This analysis suggests a potential correlation between CD8 T cell infiltration and neuronal loss with greatest magnitude of neuronal loss observed in bigenic mice that concurrently exhibit the greatest increase in CD8 T cell infiltration. I hypothesize that protective effects may be mediated via direct interactions with microglia that beneficially modulate microglial activity to facilitate beta-amyloid clearance. Conversely I hypothesize that CD8 T cells may adversely promote neuronal death in AD mouse models. My ongoing experiments include utilizing a TCRalphaKO mouse model to investigate if a genetically-mediated absence of T cells alter AD neuropathology and microglial activation state, and 10x TCR scSeq will be used to examine differing subpopulations of brain infiltrating T cells and whether these subpopulations exhibit T cell receptor clonality. These studies aim to test the overarching hypothesis that CD8 T cells induce both protective and detrimental responses within the AD brain.

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Poster

P205. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models IV

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P205.05

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

Support: Good Ventures
NIH grant NS090934

Title: Evidence that the gut microbiota regulates progression of neurodegeneration in a mouse model of tauopathy, in a sex- and ApoE isoform-dependent manner

Authors: *D.-O. SEO[1], D. O'DONNELL[1], J. STANLEY[1], N. JAIN[1], J. ULRICH[1], J. R. SERRANO[1], X. BAO[1], M. LEMIEUX[1], M. KARLSSON[1], M. MEIER[1], C. DESAI[1], J. LELWALA-GURUGE[1], K. ALVES DE LIMA[1], J. KIPNIS[1], S. S. SISODIA[2], J. I. GORDON[1], D. M. HOLTZMAN[1];
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Abstract: Alzheimer’s disease (AD) is a fatal progressive neurodegenerative disease. Mounting evidence supports that perturbations in the gut microbiota (GM) may be causally linked to amyloid-β deposition, potentially via neuroinflammation and/or disrupted metabolic homeostasis. However, the contribution of the GM to tau aggregation and accumulation, which is strongly correlated with cognitive decline in AD and other tauopathies, is poorly characterized. ApoE4 allele is the strongest genetic risk factor for late-onset AD. Here, we use a mouse model of tauopathy to explore the hypothesis that the GM regulates tau-mediated neurodegeneration in a sex- and ApoE isoform- dependent manner. Male and female P301S tau transgenic mice were crossed with human ApoE knock-in mice (ApoE3 or ApoE4) or ApoE knockout mice (EKO) to generate P301S:ApoE3/E3, ApoE4/E4, or P301S/EKO mice (abbreviated TE3F, TE4F, and TEKO, respectively). The GM in each group was perturbed by gastric gavage of an antibiotic cocktail (ABX) from postnatal days 16-22; controls were gavaged with water. Mice were maintained in a specified pathogen-free facility and fed a standard mouse chow diet *ad libitum* until euthanasia at 9.5 months. In addition, germ-free male and female TE4F mice were characterized. Conventionally-raised (CONV-R), SPF male TE3F and TE4F mice not exposed to ABX showed significant hippocampal atrophy compared to their P301S counterparts lacking a functional ApoE gene (TEKO). However, CONV-R ABX-treated TE3F mice had significantly milder hippocampal atrophy compared to water-treated control. ABX treatment of TE4F animals was also associated with milder hippocampal atrophy, although the effect did not achieve statistical significance. There were no effects in CONV-R TEKO mice treated with ABX. Remarkably, these phenotypic effects of ABX treatment were not observed in females. Moreover, germ-free TE4F mice showed a marked decrease in brain atrophy compared to conventionally-raised animals; this was true for both sexes. We have examined immunological and signaling mechanisms that modulate the observed neurodegeneration. Our results indicate that tau-mediated neurodegeneration occurs in an ApoE- dependent manner and is influenced by the gut microbiota and sex.

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Poster

P205. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models IV

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P205.06

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

Title: Microgliosis and peri-plaque tau pathologies following chronic TREM2 activation

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Abstract: Alzheimer's disease (AD) is the most common form of dementia and characterized by memory loss and progressive decline in cognitive function which ultimately leads to death. Variants in the triggering receptor expressed on myeloid cells 2 (TREM2) are associated with increased risk for sporadic, late-onset AD. Studies have shown that loss of TREM2 function impairs the microglial response to amyloid-β (Aβ) plaques which correlates with increased peri-plaque neurite damage. Importantly, microglia and TREM2 lay at the critical intersection of Aβ and tau pathologies in AD and it is hypothesized that loss of plaque-associated microglia in TREM2 variant carriers increases AD-risk and disease progression via increasing susceptibility to tau-seeding and spread. Through use of a 5XFAD amyloid mouse model induced to display neuritic plaque (NP) tau pathology, this study looks at the effect of chronic activation of TREM2 on microglia and the development of peri-plaque tau pathologies. 30 male and 30 female 5XFAD amyloid mice at 6 months of age were injected in the dentate gyrus and overlying cortex with sarkosyl insoluble tau isolated from human AD brain tissue and at this time received intraperitoneal injection of a murine agonist TREM2 antibody or a murine IgG control antibody

weekly for 3 months. At 9 months of age, we are assessing microglial and astrocytic activation, density and clustering around Aβ plaques within the cortex and hippocampus of these mice. Additionally, we are assessing the Aβ plaque burden and compaction along with assessing NP tau pathology after treatment. Finally, we are assessing neuritic dystrophy through BACE1 expression. We hypothesize that treatment with the TREM2 antibody will increase microglial density and clustering around Aβ plaques, decrease neuritic dystrophy and decrease NP tau pathology. Our study will provide further insight on TREM2 as a targetable treatment option in AD.

Disclosures: N. Jain: None. **I. Tassi:** None. **J.D. Ulrich:** None. **D.M. Holtzman:** Other; Co-founder, C2N Diagnostics LLC. Scientific advisory boards/consulting: Genentech, Denali, C2N Diagnostics, Cajal Neurosciences, Takeda, Casma, Eli Lilly.

Poster

P205. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models IV

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P205.07

Topic:

Support: Alzheimer’s Research UK (ARUK) grant TVPG 72499
UK Medical Research Council (MR/N013867/1)
Cure Alzheimer's Fund

Title: Alterations of synaptic transmission at plaque-associated regions and glial responses in the presence of the TREM2^{R47H} mutation in APP knock-in mice

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Abstract: The role of glia in disease progression has become a central focus for Alzheimer’s disease research. Studies have shown that both microglia and astrocytes cluster around amyloid plaques, where microglia may phagocytose synapses. Therefore, this work aimed to explore if this is a protective mechanism to limit plaque-associated dysfunction and how impaired microglial function, as conferred by the TREM2^{R47H} mutation, affects synaptic function and glial clustering in plaque regions. Voltage-clamp recordings were made in brain slices from *APP*$^{NL-F}$ and *APP*$^{NL-F}$/*TREM2*^{R47H} mice. In particular, unitary excitatory postsynaptic currents (uEPSCs) were evoked at CA3-CA1 synapses in the hippocampus of male and female mice with moderate plaque pathology. Post-hoc analyses were performed to assess how synaptic activity is altered depending on whether plaques were present between the stimulation site and the recorded cell.

Furthermore, hippocampal sections obtained from the contralateral hemisphere of the same mice were stained using immunohistochemistry to examine the distribution of $IBA1^+$ microglia and $GFAP^+$ astrocytes in relation to their distance from plaques. While both *APP*$^{NL-F}$ and *APP*$^{NL-F}$/*TREM2*^{R47H} mice showed a tendency for increased glutamate release when a plaque was detected ($p=0.058$), reductions in uEPSC amplitudes in plaque conditions were found only in *APP*$^{NL-F}$ mice ($p<0.05$). Thus, the effects on amplitude but not release are dependent on microglial function. Interestingly, *APP*$^{NL-F}$/*TREM2*^{R47H} mice exhibited reduced microglial density in regions with higher plaque density and specifically immediately in the vicinity of plaques ($p<0.0001$). This was more prominent in the vicinity of plaques larger than $100\mu m^2$. These mice also demonstrated higher overall plaque load than *APP*$^{NL-F}$ mice ($p<0.01$). Furthermore, although these mice did not exhibit different astrocytic density in the whole hippocampus, astrocytic area was significantly reduced near plaques in *APP*$^{NL-F}$/*TREM2*^{R47H} mice compared to *APP*$^{NL-F}$ mice. This effect was lost further away from plaques. Altogether, this study demonstrates that functioning TREM2 is necessary for some but not all plaque-associated effects on synaptic transmission which may be dependent on appropriate microglial and astrocytic response to plaques. Such compromised microglial function could potentially contribute to the spread of damage in neuronal networks.

Disclosures: K.S. Vitanova: None. **R.M. Joghee:** None. **K.M. Stringer:** None. **D.M. Cummings:** None. **F.A. Edwards:** None.

Poster

P205. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models IV

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P205.08

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

Support: Cure Alzheimer's Fund
ARUK-PG2019B-018

Title: Spatial transcriptomics reveals a plaque-specific microglial expression-type dependant on Trem2

Authors: *J. WOOD[1], E. WONG[1], A. VANSCHOIACK[3], S.-L. J. PHELAN[3], R. JOGHEE[2], K. S. VITANOVA[1], A. PATHAK[4], T. TRIPATHI[1], J. HARDY[1], D. M. CUMMINGS[1], F. A. EDWARDS[1];
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Abstract: The response of microglia to accumulating amyloid-beta plaques has become a pertinent feature of Alzheimer’s disease. RNA-seq-based techniques used to describe this

response consistently report a disease-associated microglial (DAM) population characterised by an upregulation of genes associated with inflammatory and phagocytotic processes. However, finding whether the plaque association of microglia is paralleled by a DAM-like expression signature has been limited by the lack of spatial context and absence of cell-specificity when employing bulk RNA-seq. To overcome this problem, we have undertaken a preliminary study using the GeoMx Digital Spatial Profiler (DSP; NanoString, Seattle WA) to explore the hypothesis that genes classically associated with DAM will be upregulated specifically in plaque-associated microglia and reduced upon the introduction of the R47H mutation in the microglial hub gene *Trem2*.
Paraffin-embedded hippocampal 8-micron sections were prepared from 18-month App^{NL-F} knock-in mice, App^{NL-F} knock-in mice harboring the *Trem2*-R47H mutation (both n=4), and age-matched wild type and *Trem2*-R47H controls (n=2-3). After immunohistochemistry was performed to identify microglia, astrocytes and plaques, the sections were processed by the GeoMx DSP. Whole-transcriptome RNA-probes were collected from microglia-stained plaque-contacting, peri-plaque, and away-from-plaque regions. Bulk RNA-seq was also performed using RNA collected from 18-month APP^{NL-F} knock-in mice and age-matched wild type controls (n=10-12).
GeoMx DSP analysis revealed that many DAM-associated genes were increased only in plaque-contacting or peri-plaque regions (e.g., 2.5-fold increase ($P<0.05$) in *Tyrobp* expression in App^{NL-F} plaque-contacting versus peri-plaque regions). The introduction of the *Trem2*-R47H mutation into App^{NL-F} knock-in mice induced an intermediate microglial activation state that presented a reduced expression of many *Trem2*-associated genes. Furthermore, many DAM-associated genes that were not significantly increased in our bulk RNA-seq analysis showed a significant increase in expression around plaques in the GeoMx analysis for App^{NL-F} mice.
DAM populations are not homogenous throughout a plaque burdened hippocampus but rather show spatial specificity towards plaque regions. This plaque-induced expression-type is partially impaired in microglia harbouring the *Trem2*-R47H mutation, once again revealing the importance of TREM2 in controlling microglial activation.

Disclosures: J. Wood: None. **E. Wong:** None. **A. VanSchoiack:** A. Employment/Salary (full or part-time):; NanoString Technologies. **S.J. Phelan:** A. Employment/Salary (full or part-time):; NanoString Technologies. **K.S. Vitanova:** None. **A. Pathak:** None. **T. Tripathi:** None. **J. Hardy:** A. Employment/Salary (full or part-time):; UCL. **D.M. Cummings:** A. Employment/Salary (full or part-time):; UCL. **F.A. Edwards:** A. Employment/Salary (full or part-time):; UCL.

Poster

P205. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models IV

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P205.09

Topic:

Support: Cure Alzheimer's Fund

Title: Gene expression throughout development of pathology in APPKI mice; effects of human Tau and ageing

Authors: *T. TRIPATHI*[1], A. PATHAK*[2], R. WANG[3], J. WOOD[1], S. JIANG[4], C. SIT[1], S. BAYRAM[5], D. TRABZUNI[6], D. SALIH[6], J. A. HARDY[6], D. M. CUMMINGS[1], F. A. EDWARDS[1];
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Abstract: The advances in genetics of Alzheimer's disease (AD), particularly from Genome Wide Association Studies, have been pivotal in increasing understanding of the interacting factors that influence the progression of pathology and risk of eventual onset of the disease. The development of APP knock-in (KI) mice with familial AD genes has been an essential step in improving models for AD, without problems of overexpression and random transgene insertion. Here, we present genome-wide transcriptomic analysis, from 2 to 24 months, in the hippocampus of male and female APPKI mice with and without heterozygous human Tau (KI). We correlate changes in gene expression and consider development of pathology as well as comparing male and female mice. We then assess the differences caused by the presence of human Tau and investigate development of plaques and the interaction with human Tau, to assess Aβ-induced Tau pathology.Perhaps most notably we find that introduction of human Tau heterozygously into APPKI mice, results in decrease in expression of mitochondrial genes for Complex V of the respiratory chain, particularly ATP8, with increasing age, while nuclear expressed mitochondrial genes for Complex V tend to increase in expression. We suggest that human tau inclusion changes the interaction of tau with mitochondria possibly due to increased susceptibility to phosphorylation with the increased amyloid β and ageing. These effects are being further verified and assessed using qRT-PCR and immunohistochemistry.The preliminary network analysis of gene expression identifies microglial modules correlated to pathology development in the two APP KI lines (App^{NL-F} and App^{NL-G-F}), similar to what we previously reported in transgenic mice. We see a strong upregulation of microglial genes in App^{NL-G-F} mice and a weaker effect in the later-plaque-developing App^{NL-F} mice, that we hypothesise to be related to a protective effect of the microglia. Inclusion of heterozygous human Tau results in a small decrease in this correlation of genes of microglial modules in both lines suggesting that, with the inclusion of human Tau, the protective effect of the microglia is compromised. Addition of human Tau also results in inverse correlations for modules for protein synthesis compared to APPKI mice and in App^{NL-G-F} mice inclusion of human Tau similarly affects synaptic proteins modules. Hence, RNAseq genome-wide studies of APPKI mice confirm findings from previous transgenic studies, particularly for microglial modules, while inclusion of Tau causes additional alterations, not previously reported.

Disclosures: T. Tripathi*: None. **A. Pathak*:** None. **R. Wang:** None. **J. Wood:** None. **S. Jiang:** None. **C. Sit:** None. **S. Bayram:** None. **D. Trabzuni:** None. **D. Salih:** None. **J.A. Hardy:** None. **D.M. Cummings:** None. **F.A. Edwards:** None.

Poster

P205. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models IV

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P205.10

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

Support: The federal state Saxony-Anhalt and the European Structural and Investment Funds (ZS/2016/08/80645)

Title: Upregulated synaptic remodeling in response to light-induced engagement of microglia under inflammatory conditions in adult mice

Authors: *C. CANGALAYA[1], W. SUN[2], A. GODFRIED[4], S. WEGMANN[3], S. STOYANOV[2], K.-D. FISCHER[4], A. DITYATEV[5];
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Abstract: Microglia, the resident macrophages of the central nervous system (CNS), play a crucial role in immune defense and in maintaining CNS homeostasis. Microglia continuously scan synapses, but active synaptic remodeling by these cells is rarely directly detected in mature healthy brains. Hence, we developed an approach to efficiently attract microglia to specific synaptic sites using targeted laser photoablation of single synapses. The photodamage temporally and focally elevated the frequency and duration of microglia-neuron interactions, and dramatically increased both the turnover of dendritic spines and presynaptic boutons as well as the appearance of new filopodia originating from spine heads or boutons. Microglia depletion experiments confirmed that elevated spine turnover and the generation of presynaptic filopodia are microglia-dependent processes. Since microglia activation plays an important role in neurodegeneration, we evaluated microglial dynamics in the context of systemic inflammation and AD pathology in adult mice. Systemic and local acute activation of microglial cells (with lipopolysaccharide or sarkosyl-insoluble fraction derived from AD patient brain) increased photodamage-related synaptic remodeling and generation of filopodia as compared to resting microglia. Noteworthy, the synaptic loss observed after both types of immune challenges was preceded by prolonged microglia contact and was partially mediated by microglia phagocytosis. Our results provide direct evidence that activated microglia is not only more active in pruning dendritic spines through phagocytosis but also actively promotes the formation of new spines.

Interestingly, both immune challenges decreased surveillance of synapses by increasing microglia contact duration with a fewer number of synapses under baseline conditions. Together, these results indicate that microglia play a critical role in synaptic remodeling which is modulated by their activation state in adult brains, and uncover new aspects of how the activation of the immune system may impact synaptic connectivity and cognitive function.

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Poster

P205. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models IV

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P205.11

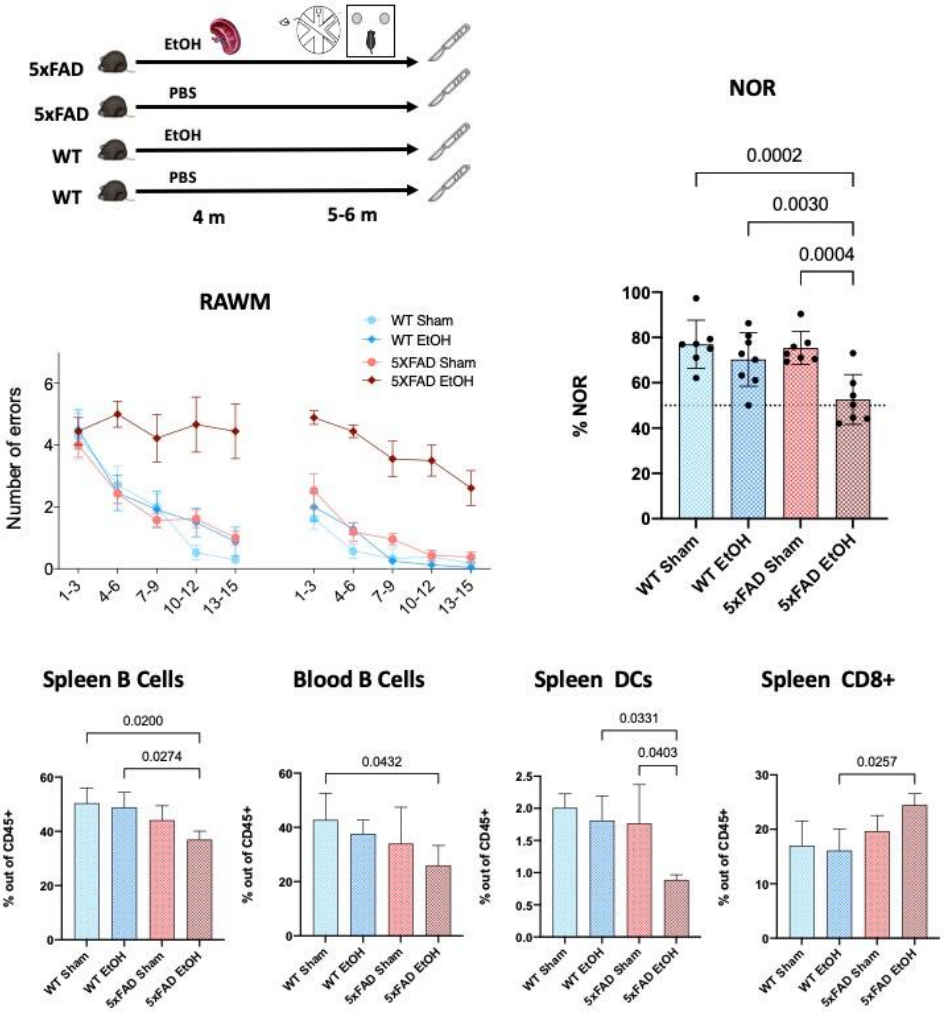
Topic: C.02. Alzheimer's Disease and Other Dementias

Title: Loss of spleen sympathetic innervation accelerates cognitive loss in Alzheimer’s disease animal model

Authors: *T. CROESE[1], M. ARAD[2], S. PINCHUK[1], G. CASTELLANI[1], J. M. PERALTA RAMOS[1], S. SUZZI[1], M. SCHWARTZ[1];
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Abstract: The immune system and the central nervous system are each in direct contact with the other organs of the body, although their interconnection is only starting to be appreciated. The periventricular nucleus of the hypothalamus (PVN) and the central nucleus of the amygdala (CeA) were shown to have an influence on immune responses via their innervation of the spleen, the major secondary lymphoid organ, through their connections with the Nucleus Tractus Solitarii (NTS) and the Vagus nerve. Both the PVN and the CeA suffer neuronal loss and Tau pathology during neurodegenerative disorders such as Alzheimer’s Disease (AD), nevertheless, to the best of our knowledge, the impact and consequences of impairment in this pathway in AD has never been studied. To test the role of splenic innervation in animal models of AD (5xFAD) and WT mice, we adapted a surgical denervation protocol treating splenic nerve plexuses with alcohol before they enter the spleen, selectively ablating the innervation of the spleen without affecting other organs. Sham-operated mice were used as controls in both groups. Subsequently, we tested the cognitive performance (Novel Object Recognition - NOR; Radial Arm Water Maze - RAWM) of all mice (4 groups, 8 mice per group), and we studied their immune landscape in both the spleen and blood through high-dimensional single-cell mass cytometry (CyTOF). Loss of the sympathetic innervation of the spleen significantly accelerated cognitive loss in 5xFAD but not in WT mice (NOR: $F(3, 24)=8.04$, $p<0.001$. RAWM: $F(3, 24)=32.02$, $p<0.001$). The cognitive loss was associated with significant immune alterations that were more pronounced in 5xFAD mice compared to WT mice. Specifically reduction of splenic and circulating B cells

(*p's*<0.05), reduction of splenic Dendritic cells (*p<0.05*), and increase in splenic CD8+ T cells (*p<0.05*). Overall, these results show that the integrity of the spleen and the immune system play an important role in the ability to cope with AD, and suggest that AD-related deterioration in the CeA and PVN might lead to immune dysfunction that further accelerates the disease.



Disclosures: T. Croese: None. **M. Arad:** None. **S. Pinchuk:** None. **G. Castellani:** None. **J.M. Peralta Ramos:** None. **S. Suzzi:** None. **M. Schwartz:** None.

Poster

P261. Parkinson's Disease: Subthalamic Nucleus and Deep Brain Stimulation

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P261.01

Topic: C.03. Parkinson's Disease

Support: Ambizione Fellowship (Swiss National Science Fundation)
Parkinson Suisse

Title: Subthalamic nucleus dynamics enable real-time decoding of natural and pathological walking in Parkinson's disease

Authors: *Y. THENAISIE[1,2], K. LEE[3], C. MOERMAN[1,2], S. SCAFA[4], A. GALVEZ[5,2], M. CASTRO JIMENEZ[1], E. PIRONDINI[5], A. PUIATTI[4], G. COURTINE[2,5], J. BLOCH[2,1], E. MARTIN MORAUD[2];
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Abstract: Most patients with advanced Parkinson's disease (PD) exhibit disturbances of gait, which severely affects their independence and quality of life. A commonly used therapy, deep brain stimulation (DBS) of the subthalamic nucleus (STN), has limited efficacy on severe gait deficits. We reasoned that the identification of STN neural dynamics during walking could guide the design of stimulation protocols specifically addressing gait deficits.
To deconstruct the neural dynamics underlying walking, we designed a well-controlled paradigm restricted to single joints of the legs. We recorded the local field potentials from DBS electrodes implanted in the STN of 20 PD patients as they produced varying isometric forces restricted to a single joint. Isometric force production led to low (~13-20Hz) and high beta (~20-35Hz) desynchronizations of STN LFPs that were time-locked to effort initiation, adaptation and termination, followed by a beta rebound. These modulations were present during passive and active movements. Surprisingly, these modulations were not only present in the STN ipsilateral to the active legs, but also on the contralateral side. Maintaining stronger efforts involved longer desynchronizations. This understanding allowed us to design a decoder based on a random-forest classifier that predicted the level of effort online.
We then leveraged these findings to design comparable conditions during gait. We found that gait initiation, adaptation and termination were marked by beta desynchronizations and could be decoded efficiently by our classifier. Additionally, the STN encoded changes in the length of the step, which allowed decoding of left versus right gait phases. Lastly, we identified increase in low beta power during freezing of gait, which supported the design of a freezing episode decoder.
These results may guide the decoding of abnormal gait patterns in PD, such as asymmetry,

shuffling steps or freezing of gait. Such biomarkers are critical for the development of adaptative DBS protocols targeting gait disorders.

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Poster

P261. Parkinson's Disease: Subthalamic Nucleus and Deep Brain Stimulation

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P261.02

Topic: C.03. Parkinson's Disease

Support: NINDS T32MH020068 (P.M.L.)
Doris Duke Clinical Scientist Development Award #2014101 (W.F.A.)
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Neurosurgery Research and Education Foundation grant (W.F.A.)
Lifespan Norman Prince Neurosciences Institute
Brown University Robert J. and Nancy D. Carney Institute for Brain Science
NIH Office of the Director grant S10OD025181

Title: Alternating Neurophysiological States representing Parkinson's Tremor, Bradykinesia, and Optimal Motor Performance in the Subthalamic Nucleus and Sensorimotor Cortex

Authors: *P. LAURO[1], S. LEE[2], D. E. AMAYA[1], D. LIU[1], U. AKBAR[3], W. F. ASAAD[4]; [2]Neurosci., [3]Neurol., [4]Neurosurg., [1]Brown Univ., Providence, RI

Abstract: Neurophysiological biomarkers of disease expression can be used to monitor disease progression, assess therapeutic efficacy, and enable effective closed-loop neuromodulation. In the case of Parkinson's disease (PD), motor manifestations are heterogeneous and asynchronous. Thus, we examined neural activity in the subthalamic nucleus (STN) of 32 subjects (4 female, 47-78 years old) with PD to decode tremor and bradykinesia, two distinct, cardinal signs of this disease, and to discriminate these states from asymptomatic periods of behavior using a rigorous, quantitative visual-motor task. We also obtained electrocorticography along sensorimotor cortex from 10 of these subjects. Using support vector regression (SVR) with depth and electrocorticography signals, we found that tremor and bradykinesia had distinct, nearly opposite neural signatures, and that effective motor control without tremor or bradykinesia displayed unique, differentiating features.
Within the STN, we discovered that optimal tremor and bradykinesia decoding models leveraged distinct frequencies (theta-alpha; 3-12 Hz v. beta-gamma; 20-30/60-100 Hz, respectively) within the local field potential spectrum. We also found that each motor sign was more strongly

represented in a distinct subregion of the STN, with optimal tremor decoding sites (x = +14, y = +13, z = −5) found to be dorsolateral to optimal bradykinesia decoding sites (x = +11, y = +13, z = −8).
In addition, tremor and bradykinesia decoding from cortical recordings exhibited superior decoding performance relative to STN recordings for both tremor (ECoG v. STN, r = 0.275±0.226 v. 0.204±0.236, p = 0.002, Mann-Whitney U test) and bradykinesia (r = 0.249±0.222 v. 0.109±0.175, p < 0.001). While cortical decoding models for tremor and bradykinesia used similar frequency bands (beta and gamma), their model weights were applied in an opposing fashion between tremor and bradykinesia.
To dissociate optimal motor performance from symptomatic states, a measure of ”good performance“ was also calculated as the absence of tremor and bradykinesia for each patient. SVR models decoding good performance revealed distinct gamma frequency activity for both STN (30-45 Hz) and cortical (45-55 Hz) recordings.
Overall, tremor and bradykinesia were better decoded from different portions of the STN, and cortical decoding accuracy generally exceeded subcortical decoding. While tremor and bradykinesia exhibited opposing frequency profiles of spectral activity, optimal motor performance correlated with specific gamma frequencies. These results provide a roadmap to leverage real-time neurophysiology to understand and treat PD.

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Poster

P261. Parkinson's Disease: Subthalamic Nucleus and Deep Brain Stimulation

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P261.03

Topic: C.03. Parkinson’s Disease

Support: SK Foundation

Title: Deep brain stimulation influences networks rather than individual neuron activity in the cortico-basal ganglia motor circuit

Authors: *A. C. GUEST[1], D. GRAHAM[2], H. SHILL[2], F. PONCE[2], B. GREGER[3];
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Abstract: Deep brain stimulation (DBS) is a well-established treatment for Parkinson’s disease (PD) and other movement disorders, where stimulation is delivered to particular anatomical targets in the brain to alleviate symptoms of the disease. Our goal is to investigate the network

dynamics of the cortico-basal ganglia circuit and local circuits within the subthalamic nucleus (STN) when applying DBS. This project is part of a larger effort to understand the neurophysiological mechanism of DBS, and how DBS changes motor processing in the human brain. There is a gap in our knowledge regarding if DBS affects the brain on a cellular versus a network level (McIntyre et al. 2004; Lozano et al. 2019). Here, we examine individual neurons, local circuits, and network relationships in the STN and cortex with DBS. Patients with PD underwent neurosurgery for DBS electrode placement. Intraoperatively, we recorded APs and LFPs from a microelectrode placed in STN, 2 mm anterior to the DBS lead. We simultaneously recorded LFP in the premotor cortex using a micro-electrocorticography (micro-ECoG) grid. DBS was performed in the anesthetized patient at various frequencies (30, 70, 140, and 250 Hz). Statistical significance was tested with ANOVA or t-tests and used a time-jittered "AP"-aligned LFP as a control. In our preliminary results, DBS does not change the firing rate of neurons in the STN. The LFP in the STN aligned on APs from a neuron in the STN shows increased power in the alpha frequency band when stimulated at a clinically relevant frequency (140 Hz). The cortex has a complex relationship with the neurons on the STN on subcentimeter scale, but it does not appear to be affected by DBS. Our results provide insight into the effects of DBS on neural tissue. This data suggests that DBS acts at the network level because of the lack of effect on STN neuron firing and the changes in AP-LFP relationship. Examining the cortex on a spatial scale relevant to cortical activity is critical to understanding the role of the cortex in the mechanism of DBS. LFPs on the cortex show a complex relationship with the STN and in response to DBS, perhaps reflecting multiple anatomic routes for stimulation propagation. Determining the electrophysiological changes in neural circuits with DBS will advance our mechanistic understanding of DBS in the cortico-thalamic-basal ganglia circuit and provide insight into DBS and neural pathology.

Disclosures: A.C. Guest: None. **D. Graham:** None. **H. Shill:** None. **F. Ponce:** None. **B. Greger:** None.

Poster

P261. Parkinson's Disease: Subthalamic Nucleus and Deep Brain Stimulation

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P261.04

Topic: C.03. Parkinson's Disease

Support: NIH Grant T32NS007480-20
NIH Grant K23NS097576
NIH Grant 1P50NS098685

Title: Deep brain stimulation-induced local evoked potentials can guide intraoperative targeting and postoperative programming in Parkinson's disease.

Authors: *E. OPRI[1], F. ISBAINE[2], N. AU YONG[2], J. T. WILLIE[3], N. M. BOULIS[2], T. WICHMANN[1], R. E. GROSS[2], S. MIOCINOVIC[1];
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Abstract: Deep brain stimulation (DBS) of the subthalamic nucleus (STN) has become standard therapy for patients with Parkinson's disease (PD). Both accurate surgical implantation and optimal postoperative programming of the DBS device are required to realize the full therapeutic benefit, determining which parameters provide the best symptom suppression and the fewest side effects. This process is a time consuming trial and error approach, relying on clinical intuition and repeated patient exams. We studied whether the placement of leads and postoperative programming could instead be guided by measuring DBS local evoked potentials (DLEP) during the implantation surgery. DLEP is a strongly stereotyped stimulation-induced oscillatory activity measured from non-stimulating DBS lead contacts. We hypothesized that contacts whose stimulation produces the largest DLEP amplitude would be localized in the dorsal STN and would have the best therapeutic effect.We recorded intraoperatively DLEPs from leads implanted in the STN using low-frequency (1 and 3 mA, 10 Hz, 12 s blocks) stimulation in 16 patients with PD. We developed a post-processing artifact-suppression pipeline to reliably recover DLEP activity from a commercially available clinical amplifier (Neuro Omega), facilitating the deployment in routine surgery. DLEP activity was recovered after each stimulation pulse with multi-epoch averages. DLEP amplitude was estimated as the root-mean-square within 4-20ms after the pulse. Positions of the stimulating and recording contacts were determined from microelectrode mapping. We also recorded spontaneous local field potential activity at each contact to calculate peak beta power (within 13-30Hz). We surveyed clinical postoperative records to determine therapeutic efficacy of each contact (at initial programming and up to 1 year after surgery).We demonstrate that our pipeline reliably recovers DLEP from noisy recordings, due to the brief saturation recovery of the amplifier. The DLEP amplitude was indicative of the postoperatively determined therapeutic efficacy of the stimulating contact ($p<0.01$) and stimulating contact location, as it was the highest within the dorsal STN (in vs out-STN: $p<0.05$; dorsal vs ventral STN: $p<0.0001$). In contrast, contact's ability to record DLEP (as opposed to elicit DLEP) was not predictive of its location (in vs out-STN: $p>0.19$; dorsal vs ventral STN: $p>0.08$) or clinical efficacy ($p>0.5$). Beta power was less predictive of the location (in vs out-STN: $p<0.05$; dorsal vs ventral STN: $p>0.4$) or clinical efficacy ($p>0.2$). In conclusion, DLEP analysis is a promising method to guide lead placement and postoperative programming.

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Poster

P261. Parkinson's Disease: Subthalamic Nucleus and Deep Brain Stimulation

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P261.05

Topic: C.03. Parkinson's Disease

Support: NIH Grant NS115032-01A1

Title: Local Cell-Specific Pallidal Deep Brain Stimulation Ameliorates Parkinsonian Motor Deficits

Authors: *C. YU, H. LOUGHLIN, C. LOOMAN;
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Abstract: Deep brain stimulation (DBS) is an established therapy for the motor symptoms of advanced Parkinson's disease (PD). The globus pallidus interna (GPi) is a commonly used anatomical target and clinically effective for DBS in PD therapy. The therapeutic effects of GPi DBS are frequency-dependent; high frequencies relieve PD motor symptoms, while low frequencies are not effective. Despite well-established clinical benefits, how GPi DBS exerts its therapeutic effects remains unclear. Here, we performed optogenetic stimulation in the entopeduncular nucleus (EP, rat homologue of GPi) using an ultrafast opsin (Chronos) in a unilateral 6-OHDA-lesioned rat model of PD. The Chronos construct was packaged into an adeno-associated virus serotype 5 (AAV5) under the control of the human synapsin neuron-specific promoter. We quantified the behavioral effects of different frequencies of optogenetic GPi DBS on motor deficits using circling behavior and adjusting steps test. We conducted single-unit recordings in GPi and ventral lateral motor thalamus (VL) to quantify changes in neural circuit activity during 130Hz optogenetic GPi DBS in the urethane-anesthetized PD rats (1.2g/kg, subcutaneous). We found that optogenetic stimulation of GPi local neurons exhibited a strong dependence on stimulation frequencies; high-frequency DBS (130Hz) alleviated pathological ipsilateral turning and corrected abnormal forelimb stepping while low frequency (20Hz) DBS was ineffective. Optogenetic activation of GPi at 130 Hz increased (45%) or decreased (38%) neural activity in the GPi. Similarly, neurons in the VL exhibited both increases (48%) and decrease (29%) in firing rate during 130 Hz optogenetic GPi DBS. Further, we conducted a spectral analysis of single-unit firing times to quantify the effects of optogenetic DBS on oscillatory activity. We found that GPi neurons (75%) exhibited decreased beta-band oscillatory activity (13-35Hz) during 130 Hz optogenetic GPI DBS. Similarly, the majority of VL neurons (71%) exhibited a reduced oscillatory peak in the beta band during 130 Hz optical DBS. Our results suggest that the neural firing rates within GPi and VL circuits were differentially modulated and could not explain the frequency-dependent behavioral effect of GPi DBS. Rather, high-frequency optogenetic GPi DBS could ameliorate parkinsonian motor symptoms through reduction of pathological oscillatory activity in the GPi-linked neural circuit.
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Poster

P261. Parkinson's Disease: Subthalamic Nucleus and Deep Brain Stimulation

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Program #/Poster #: P261.06

Topic: C.03. Parkinson's Disease

Support: NIH P01 GM118269

Title: Deep brain stimulation in the subthalamic nucleus for Parkinson's disease can restore dynamics of striatal networks

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Abstract: Deep brain stimulation (DBS) of the subthalamic nucleus (STN) is highly effective in alleviating movement disability in patients with Parkinson's disease (PD). However, its therapeutic mechanism of action is unknown. The healthy striatum exhibits rich dynamics resulting from an interaction of beta, gamma and theta oscillations. These rhythms are at the heart of selection, initiation and execution of motor programs, and their loss or exaggeration due to dopamine (DA) depletion in PD is a major source of the behavioral deficits observed in PD patients. Interrupting abnormal rhythms and restoring the interaction of rhythms as observed in the healthy striatum may then be instrumental in the therapeutic action of DBS. We develop a biophysical networked model of a BG pathway to study how abnormal beta oscillations can emerge throughout the BG in PD, and how DBS can restore normal beta, gamma and theta striatal rhythms. Our model incorporates STN projections to the striatum, long known but understudied, that were recently shown to preferentially target fast spiking interneurons (FSI) in the striatum. We find that DBS in STN is able to normalize striatal medium spiny neuron (MSN) activity by recruiting FSI dynamics, and restoring the inhibitory potency of FSIs observed in normal conditions. We also find that DBS allows the re-expression of gamma and theta rhythms, thought to be dependent on high DA levels and thereby lost in PD, through cortical noise control. Our study shows how BG connectivity can amplify beta oscillations, and delineates the role of DBS in disrupting beta oscillations and providing corrective input to STN efferents to restore healthy striatal dynamics.

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Poster

P261. Parkinson's Disease: Subthalamic Nucleus and Deep Brain Stimulation

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Program #/Poster #: P261.07

Topic: C.03. Parkinson's Disease

Support: The authors are grateful for software code made possible via the OpenMind Consortium, an effort supported by BRAIN Initiative resource dissemination grant U24NS113637.

Title: Effects of levodopa and DBS on physiology of emotional conflict in Parkinson's disease patients implanted with a chronic sensing device

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Abstract: Parkinson's disease impairs emotion recognition and decision making due to dopaminergic denervation in mesocorticolimbic pathways. Levodopa and deep brain stimulation (DBS) have been known to modulate patient's affective states and decision making thresholds, but it remains unclear how this is indexed in neurophysiological brain signals. The objective of this study is to identify neural biomarkers and behavioral correlates of emotional conflict in Parkinson's patients with chronically implanted deep brain stimulation (DBS) electrodes and to assess the impact of levodopa and DBS on these biomarkers. Parkinson's patients (n = 4) with bilateral, chronically implanted Subthalamic Nucleus (STN) leads and a cortical sensori-motor electrocorticography 4 contact electrode strip, completed an emotional stroop task where they indicated the emotion expressed by a face while ignoring a word distractor. Patients also completed a gender-based control version of the task. Patients completed the task twice for each of the four conditions (off meds / off stim, off meds / on stim, on meds / off stim, and on meds / on stim) over 2 subsequent days. Invasive neurophysiology from the STN and motor cortex was recorded while patients performed the task using an investigational, sensing-enabled DBS stimulator. For DBS treatment conditions, patients were on their clinical monopolar DBS configuration. Across all conditions, patients made more errors for incongruent than congruent trials. The emotional stroop effect was higher for the levodopa condition than for the DBS condition with the largest stroop effect difference occurring between on/off levodopa without the presence of DBS. The control sub-task did not demonstrate an increased stroop effect when comparing levodopa on and off conditions. With DBS off, STN theta (3-7hz) power increased after stimulus presentation (300-600ms) in the on levodopa condition compared to the off levodopa condition. DBS increased STN theta power in the levodopa off condition, however theta power was decreased in the levodopa on condition. STN theta power in Parkinson's patients has been associated with conflict detection and plays a key role in decision making gating mechanisms. This study's behavioral and neurophysiological results suggest that dopamine and DBS modulate emotional conflict signals in the STN which may mediate their impact on behavior.

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Poster

P261. Parkinson's Disease: Subthalamic Nucleus and Deep Brain Stimulation

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Program #/Poster #: P261.08

Topic: C.03. Parkinson's Disease

Title: Different Effects of Dopaminergic Medications on Subthalamic Beta Bursts and Non-Oscillatory Fractal Components in Parkinson's Disease: A Longitudinal Study

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Abstract: Exaggerated beta power in the local field potentials (LFPs) recorded from the subthalamic nucleus (STN) is a hallmark of Parkinson's disease (PD). Longer durations and higher amplitudes of beta bursts are associated with motor impairment. STN- deep brain stimulation (DBS) and dopaminergic therapy attenuate beta power and shorten beta burst durations in PD patients. To date, little is known about the long-term effects of STN-DBS on beta oscillations and how it relates to disease progression after the stun effect of DBS placement has disappeared. The first aim of this study was to investigate the long-term effects of STN-DBS and levodopa on the beta band characteristics. In addition to oscillations, which are often identified as narrowband peaks in the spectrogram of the neurophysiological signals, brain activity entails a dominant fractal (non-oscillatory) component. There is ample evidence that fractal components carry critical physiological and pathophysiological characteristics closely linked to neural functions. The second aim of this longitudinal study was to separate oscillatory and fractal components, which are likely generated through different mechanisms. Here we used Irregular-Resampling Auto-Spectral Analysis (IRASA) to separate oscillatory and fractal components in the power spectrum of the STN-LFP signals and hypothesized that chronic STN-DBS and levodopa treatment not only change the oscillatory part of the beta band but also modulate the fractal component of beta-band signals. Ten patients with idiopathic PD participated in this study. Simultaneous bilateral STN-LFP recordings were conducted in five separate visits at 1, 2, 6, 12, and 18 months post-operation. Each visit was conducted over two days in the OFF and ON dopaminergic medication states. The results revealed a relatively stable benefit of dopaminergic medication for normalizing pathologic beta bursts over time with shorter burst durations and lower burst amplitudes in the ON medication compared to the OFF medication state. In contrast, fractal components were not significantly changed with dopaminergic medications. However, both the slope (exponent) and intercept (offset) of the beta band fractal components progressively increased over time. Since previous studies have shown

that global enhancement of the GABAergic transmission under general anesthetics resulted in larger slope of the fractal component, our findings suggest that long-term STN-DBS might have increased inhibition in the basal ganglia-cortical circuitries.

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Poster

P261. Parkinson's Disease: Subthalamic Nucleus and Deep Brain Stimulation

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Program #/Poster #: P261.09

Topic: C.03. Parkinson’s Disease

Support: NIH-NINDS R01NS113746
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Title: 3d-ultrastructural analysis of gabaergic and glutamatergic synapses in the subthalamic nucleus of parkinsonian monkeys

Authors: *A.-C. MARTEL[1,2], R. M. VILLALBA[1,2], T. WICHMANN[3,1,2], A. GALVAN[1,2,3], Y. SMITH[1,2,3];
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Abstract: The subthalamic nucleus (STN) is part of the indirect pathway of the basal ganglia, receiving its main glutamatergic and GABAergic innervation from the cerebral cortex and the external globus pallidus (GPe), respectively. Alterations of STN firing rates and patterns are linked to the severity of parkinsonian motor signs. Neuroplastic changes in the abundance, morphology and strength of GABAergic and glutamatergic synapses to the STN may contribute to the pathophysiology of parkinsonism. Severe cortical glutamatergic denervation of the STN has been described in primate and mouse models of parkinsonism (Mathai et al., 2015, Brain 138:946; Fan et al., 2012, JNS 32:13718). Additional changes in the strength and morphology of GPe-STN GABAergic synapses have been characterized in parkinsonian mice, but not in primates. To further characterize structural neuroplastic changes of GPe-STN terminals in parkinsonian monkeys, we undertook a quantitative morphometric 3D electron microscopy (3D-EM) analysis of these terminals and their synapses in the STN of MPTP-treated monkeys. The single block facing/scanning electron microscopy (SBF/SEM) approach combined with the *Reconstruct* software was used to address this issue. Preliminary data were collected from 55

3D-reconstructed GABAergic pallidal terminals in the STN of 2 MPTP-treated parkinsonian monkeys. The pallidal terminals were identified either by anterograde labeling from the GPe or using known ultrastructural features (large size, multiple symmetric synapses, two or more mitochondria). We collected morphometric data for each terminal, including its volume, the flat area of the post-synaptic densities (PSDs) formed by the terminal, and the number and size of mitochondria within the terminal. Our data show that the volume of GPe terminals ranged from 1.13-26.41μm^3 (mean ± SEM= 4.02 ± 0.47). Each terminal formed large and complex multi-fragmented synapses with flat areas varying from 0.04-2.67μm^2 (0.42 ± 0.05). The number of mitochondria per terminal ranged from 2-31 (5.15 ± 0.55), while the volume of individual mitochondria varied between 0.06-0.34μm^3 (0.15 ± 0.008). Studies in control monkeys are in progress. To determine the impact of the parkinsonism-associated breakdown of the cortico-STN projection on the pattern of synaptic innervation of STN neurons, 3D-EM approaches are being used to map the localization and quantify the abundance of glutamatergic and GABAergic terminals on STN dendrites in control and parkinsonian monkeys. Together, results of these studies will lead to a better understanding of the parkinsonism -associated changes of the synaptic innervation of STN neurons.

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Poster

P277. Huntington's Disease: Models

Location: Virtual Only

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Program #/Poster #: P277.01

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Evaluation of marmoset as possible non-human primate model for HTT splicing modulation studies

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Abstract: Several modalities aimed at lowering levels of pathogenic mutant HTT (mHTT) are currently in development for the treatment of Huntington’s Disease. Recently, small molecules have been identified targeting HTT mRNA splicing, that result in nonsense mediated decay (NMD) decreasing HTT mRNA and thus lowering HTT protein production. The use of orally bioavailable, brain penetrant small molecules for therapeutic intervention represents a potential advantage over other drug modalities currently in development.

One of the current small molecule splicing modulators appears to efficiently target a sequence in the human HTT gene (exon 49/50 and the intervening intron) that is poorly conserved in rodent HTT, thereby precluding the ability to directly test these compounds for on-target mechanisms (efficacy and safety) in standard rodent models.
The evaluation of a non-human primate (NHP) model would be beneficial towards progression of splicing modulating candidate molecules and assessment of possible off-target effects. Here we described the characterization of the Callithrix jacchus (common marmoset) as an NHP model given the strong similarity between marmoset and human HTT genomic sequences (95.1%) and the establishment of *in vitro* assays. Thereupon, a buccal cavity tumour and skin biopsies taken from common marmosets were used for the generation of immortalized marmoset fibroblast lines. Splicing modulation-mediated effects were assessed using a set of marmoset-specific Taqman assays to detect alternative splicing of HTT by the evaluation of exon 49-50 levels and iExon49b inclusion and additionally the effects on HTT protein levels were evaluated. Off-target effects were assessed by measurement of MADD exon 20-21 and FOXM1 exon 8-9 levels. Together the data demonstrate the evaluation of the common marmoset as model for HTT splicing modulation studies.

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Poster

P277. Huntington's Disease: Models

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Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Grant NS084298
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Title: Generation and characterization of a Human Huntingtin BAC Transgenic Mouse Model with Expanded Uninterrupted CAG repeats

Authors: *X. GU[1], J. RICHMAN[1], P. LANGFELDER[1], H.-B. WANG[1], S. ZHANG[1], N. WANG[1], L. RAMANATHAN[1], J. CANTLE[1], C. PARK[1], C. CHOI[1], M. PLACENCIA[1], F. GAO[1], L. YANG[1], M. GRAY[2], G. COPPOLA[1], G. BATES[3], S. HORVATH[1], C. S. COLWELL[4], X. W. YANG[5];
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Birmingham, AL; [3]Univ. Col. London, London, United Kingdom; [4]Psychiatry and Biobehavioral Sci., Univ. of California - Los Angeles, Los Angeles, CA; [5]Dept Psych, UCLA, Los Angeles, CA

Abstract: Generation and characterization of a Human Huntingtin BAC Transgenic Mouse Model with Expanded Uninterrupted CAG repeats

Xiaofeng Gu[1,2], Jeff Richman[1,2], Peter Langfelder[1,2] , Huei-Bin Wang[2] , Shasha Zhang[1,2], Nan Wang[1,2] , Lalini Ramanathan[1,2] , Jeff Cantle[1,2], Chang Chris Park[1,2], Christopher Choi[1,2], Mary Placencia[1,2], Fuying Gao[1,2], Lucia Yang[1,2], Michelle Gray[3], Giovanni Coppola[1,2], Gillian Bates[4], Steve Horvath[5], Christopher S. Colwell[2], and X. William Yang[1,2]

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Abstract

In Huntington's Disease (HD), the uninterrupted CAG repeat length, but not polyglutamine length, predicts the disease onset yet the underlying pathology remains unclear. Here, we developed a human mutant huntingtin (mHTT) BAC transgenic mouse with long uninterrupted CAG repeats. This model (BAC-CAG) exhibits progressive behavioral deficits, striatal mHTT aggregation, synaptic pathology and astrogliosis. Interestingly, unlike previous human *HTT* BAC transgenic models with CAA interruptions in the polyglutamine coding region, BAC-CAG mice show robust and progressive striatal somatic repeat instability and transcriptomic dysregulation. We will present our current analysis of potential toxic entities that are associated with the distinct spatiotemporal patterns of neuropathogenesis elicited by human mHTT with or without long uninterrupted CAG repeats.

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Poster

P277. Huntington's Disease: Models

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Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: CHDI Management/CHDI Foundation
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Title: Resting-state co-activation patterns (CAPs) accurately predict pre- and manifest-stage Huntington's disease in mice.

Authors: *M. H. ADHIKARI[1], T. VASILKOVSKA[1], D. PUSTINA[2], R. CACHOPE[3], H. TANG[2], L. LIU[4], I. MUNOZ SANJUAN[3], G. A. KELIRIS[1], A. VAN DER LINDEN[1], M. VERHOYE[1];
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Abstract: Huntington's disease (HD) is a neurodegenerative disorder caused by an expansion in the number of glutamine-encoding CAG repeats in the huntingtin gene. The impact of mutant huntingtin (mHTT) on the brain's functional architecture is not fully understood. To address that, we sought the identification of functional imaging markers at the pre-manifest, early manifest, and advanced manifest stages in the Q175 heterozygous (HET) mouse model of HD. Resting-state (RS) functional MRI (1200 GE-EPI volumes, TR 0.5s) was performed at 9.4T (Bruker Biospec) using the cryo coil. 18 wild-type (WT) and 19 HET mice were scanned under anesthesia (0.5% isoflurane, 0.075mg/kg medetomidine, s.c. bolus followed by 0.15 mg/kg/h s.c. infusion), at 3 (pre-manifest), 6 (early manifest), and 10 (manifest stage) months of age. At each age, a range of 2-20 RS-co-activation patterns (CAPs) was identified based on the similarity of spatial distributions of voxel-wise z-scored BOLD signals. Data from all 39 subjects were concatenated before CAP extraction, thus enabling subsequent group comparisons. We compared WT and HET groups for voxel-level activation of each CAP as well as its duration, occurrence, and the transition entropy (a measure of systematicity of transitions to other CAPs). Finally, we assessed the predictive power of CAP features to distinguish HET from WT subjects at each age with cross-validation. Most robust inter-group differences were found in two prominent CAPs, which are human analogs. Duration and occurrence of the default-mode-like network (DMLN) CAP, displaying co-activation of DMLN and co-deactivation of the lateral-cortical network (LCN), and of its anti-correlated pair, the LCN CAP, were reduced in the HET group at the manifest stage. At the pre-, and early- manifest stages, HET animals showed lower occurrence and duration for LCN and DMLN CAP respectively. DMLN CAP's transition entropy was significantly reduced in the HET group at the manifest stage. While their durations and occurrences performed significantly better than the chance level accuracy of 50% in predicting the animal identity, the classification accuracy using voxel-wise activations of CAPs was significantly higher at nearly 80% at all stages. Our results suggest that the impact of mHTT on brain function is borne by biologically and translationally relevant transient states. Further, we find that RS-CAPs are powerful candidates for an early biomarker of Huntington's disease.

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Poster

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Topic: C.04. Movement Disorders other than Parkinson's Disease

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Title: Behaviour characterization of the Q175/B6 Huntington's disease mouse model

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Abstract: Q175/B6 is a knock-in mouse model of Huntington’s disease (HD), a neurodegenerative disorder characterized by chorea, loss of motor control and cognitive deficits. The primary site of neurodegeneration in HD is the striatum, a brain region important for motor learning. Studies have found hypoactivity and abnormal rearing behaviours in older HD mice during the open field. Other studies have revealed motor and cognitive deficits in Q175 mice on the accelerating rotarod and water T-maze tasks at around 10 months of age. This study aims to investigate behavioural changes in both 2-month-old (presymptomatic) and 10-month-old (symptomatic) Q175 mice while conducting more detailed analyses on the specific patterns of behaviour using the DeepLabCut tracking software and the new Behavioral Segmentation of Open Field in DeepLabCut (B-SOiD) machine learning program. 10-month-old Q175 mice showed differences in the frequency of locomotor, sniffing, grooming, and rearing behaviours as compared to wild-type (WT) during the open field. The older cohort also showed motor deficits on the rotarod, with a reduced latency to fall and a greater number of foot slips as analyzed with DeepLabCut. In the water T-maze, 2-month-old Q175 mice took significantly longer than WT to reach the hidden platform during the reversal phase. However, in the older group, only those that used a striatum-dependent response learning strategy during training showed a higher latency to reach the platform in both training and reversal phases. These findings reveal that Q175 mice show notable motor learning deficits at 10 months of age, as well as reduced exploratory behaviour and potentially increased anxiety-like behaviours in the open field. Our comparison between the two age groups suggests that younger Q175 mice may show early signs of impairment in cognitive flexibility, and that the specific strategy used during motor learning may be more relevant at the older age. Future studies that examine striatal signalling during performance of these tasks are needed for evaluation of potential therapeutic treatments in HD.

Disclosures: J. Cheng: None. **E.T. Koch:** None. **L.A. Raymond:** None.

Poster

P277. Huntington's Disease: Models

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P277.05

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: CHDI Foundation

Title: Preclinical behavioral assessments of systemic, allele-specific transcriptional repression of mutant huntingtin as a therapeutic strategy for Huntington’s disease

Authors: *L.-Y. ZHANG, A. Y. KIM, I. GILDISH, S. ARONI, J. F. CHEER;
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Abstract: Huntington’s disease (HD) is an inherited monogenic progressive neurodegenerative disorder caused by a polyglutamine (CAG) expansion in the huntingtin gene. Resultant striatal and cortical dysfunction are the hallmark neuropathological features underlying dyskinesia (e.g. chorea) and akinesia at later stages. In addition, motivational and cognitive deficits commonly manifest concurrently with, or preceding, motor dysfunction. A recent study reported that engineered zinc finger protein transcription factors (ZFP-TFs), tuned to selectively target pathogenic CAG repeats for allele-selective repression, when locally administered into the striatum, efficiently suppressed mHTT production and ameliorated transcriptional and electrophysiological deficits in full length heterozygous knock-in Q175 mice. Expanding on these findings, we postulated that the systemic delivery of ZFP-TFs under the control of a synapsin promoter would permit broader transduction and consequent suppression of mHTT throughout the central nervous system. However, the efficacy and tolerability of systemic ZFP-TF-mediated suppression of mHTT has yet to be elucidated. We thus optimized systemic delivery of AAV_PHP.eB packaged ZFP-TFs, with its control lacking the ZFP DNA-binding domain, in 3-month and 6-month old male Q175 mice via retro orbital injection. We assessed the tolerability of ZFP-TFs by monitoring body weight, temperature, locomotor function in an open-field arena, and anxiety-like behavior in the elevated plus maze. Additionally, 3-month old WT mice treated with ZFP-TFs were trained on a fixed-ratio (FR) 5 and progressive ratio (PR) schedule of reinforcement. Here, we show that ZFP-TF administration induced a significant decrease in bodyweight in both WT and Q175 mice across age that led to an increase in mortality in both Q175 and WT animals. Mice of both genotypes failed to demonstrate any significant differences in locomotor function across viral treatment or age. However, both 6-month old WT and Q175 mice treated with ZFP-TFs exhibited a robust decrease in time spent in the closed arms in the elevated plus maze, suggesting that ZFP-TFs exert a non-specific, anxiolytic effect on all animals across the studied age range. WT mice treated with ZFP-TFs acquired significantly

fewer rewards in both FR5 and PR tasks, indicating that ZFP-TFs compromise reward-seeking behavior by a mechanism unrelated to mHTT suppression. Our findings suggest that further validation of the tolerability of systemically administered ZFP-TFs will critically inform preclinical development of this DNA-targeting approach to ameliorate HD pathogenesis.

Disclosures: L. Zhang: None. **A.Y. Kim:** None. **I. Gildish:** None. **S. Aroni:** None. **J.F. Cheer:** None.

Poster

P277. Huntington's Disease: Models

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Program #/Poster #: P277.06

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: John G. Kulhavi Professorship in Neuroscience at CMU
The Field Neurosciences Institute
Central Michigan University Undergraduate Research and Creative Endeavors Grant

Title: Ovine-sourced GM1 ganglioside treatments to increase health and longevity in the R6/2 mouse model of Huntington's disease

Authors: *N. FETTINGER[1,3], S. KONERU[1,3], M. R. RESK[1,3], P. OTERO[1,3,4], J. ROSSIGNOL[1,3,4], G. L. DUNBAR[1,3,2,5];
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Abstract: Huntington's disease (HD) is a genetically inherited progressive neurodegenerative disease caused by a mutation in the trinucleotide repeats of cytosine, adenine, and guanine (CAG) of the huntingtin (*HTT*) gene. The mutation in the *HTT* gene increases the number of CAG repeats, leading to an increased production of the mutant huntingtin protein. Healthy individuals typically exhibit 16 to 20 CAG repeats on the *HTT* gene, while those with HD exhibit 35 or greater CAG repeats, with an increase in CAG repeats corresponding to an increased severity in HD symptoms, including motor and cognitive declines, involuntary movements, weight loss, muscle loss, and a shorter lifespan. Currently, there is no treatment for Huntington's disease. The R6/2 mouse model of HD has a mutated exon 1 of the human huntingtin gene that exhibits 160 CAG repeats, serving as severe model for individuals with HD. R6/2 mice display similar symptoms to HD patients, such as severe weight loss, muscle loss, deficits in motor function, and reduced lifespan. Individuals with HD show a decrease in GM1 ganglioside levels,

severely affecting neurons and ultimately leading to a shorter lifespan in the affected individual. Treatment of mouse models of HD with bovine-sourced GM1 gangliosides leads to a decrease in disease symptoms. Bovine-sourced GM1 gangliosides are extracted from cow brains, while ovine-sourced GM1 gangliosides are extracted from sheep brains. Ovine-sourced GM1 gangliosides are one-fourth the cost and thus may serve as a cost-efficient alternative to bovine-sourced GM1 gangliosides. The aim of this project was to determine the effectiveness of ovine-sourced GM1 ganglioside treatment on R6/2 mouse models by studying survivability and quality of life of the mouse model. The results of our study indicated that HD mice treated with ovine-sourced GM1 ganglioside showed a significant increase in weight over their lifespan compared to bovine-sourced GM1 ganglioside treated HD mice and the control HD group. Ovine-sourced and bovine-sourced GM1 ganglioside-treated HD mice had a prolonged lifespan of nine and twelve days, respectively, compared to vehicle-treated HD mice. *Support for this study was provided by the Neuroscience program, College of Medicine, and the John G. Kulhavi Professorship in Neuroscience at CMU, and the Field Neurosciences Institute.*

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Poster

P277. Huntington's Disease: Models

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Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P277.07

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: Funds from the John G. Kulhavi Professorship in Neuroscience at CMU
The Field Neurosciences Institute
Central Michigan University graduate grant

Title: Comparison of ovine and bovine sources of GM1 ganglioside for treating behavioral deficits in the R6/2 mouse model of Huntington’s disease

Authors: S. KONERU[1,3], N. WEDSTER[1,3], M. RESK[1,3], N. FETTINGER[1,3], C. MALKOWSKI[1,3], K. OLSON[1,3], O. KHACHERIAN[1,3], A. POUDEL[1,4], E. LAUZON[2,5], N. SHARMA[2,6,7], M. SANDSTROM[1,8], J. ROSSIGNOL[1,3,10], G. L. DUNBAR[1,3,9];
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Abstract: Huntington's disease (HD) is an autosomal dominant neurodegenerative disease caused by mutations in the Huntington (*HTT*) gene containing a long polyglutamine (CAG) stretch. This mutation leads to excessive production and accumulation of mutant huntingtin protein (mHTT) in the brain. This excessive mHTT protein accumulation causes motor, psychiatric, cognitive impairments and, ultimately, neuronal death. Currently, no effective treatment has been approved for HD. However, a promising line of research in therapeutics for HD, involves the use of GM1 ganglioside, as levels of GM1 are reduced in HD patients, which is thought to contribute to the disease progression. GM1 ganglioside is a sialic acid-containing glycosphingolipid that is found abundantly in the outer leaflet of the neuronal membrane in the brain. The initial source for GM1 came from bovine brains, but new sources, including synthetic as well as those obtained from the ovine brain, have been developed subsequently. Treatments with synthetic- and bovine- sourced gangliosides have been shown to reduce motor dysfunction in HD rodent models. However, the efficacy of treatment using ovine-sourced GM1, which is much more economical, has yet to be tested. The goal of this study was to compare the efficacy of ovine-sourced GM1 with that obtained from bovine sources on the motor and cognitive dysfunctions in the R6/2 mouse model of HD. In the current study, we used intraventricular osmotic pumps to deliver the different-sourced GM1 gangliosides for four weeks in five-week-old R6/2 mice. Rotarod-, open field-, catwalk-, Barnes maze- and novel-object-recognition- tasks were used to measure motor and cognitive abilities in the treated and non-treated mice. Results indicated that ovine-sourced GM1 gangliosides significantly reduced behavioral deficits in R6/2 mice on the rotarod-, open field-, catwalk-, but not on Barnes maze-, and novel-object-recognition- tasks, compared to vehicle-treated and bovine-sourced-GM1-treated R6/2 mice.

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Poster

P277. Huntington's Disease: Models

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Program #/Poster #: P277.08

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Grant 1R21NS104320-01A1

Title: Immortalized Striatal Precursor Neurons from Huntington's disease patient-derived iPS cells as a platform for target identification and drug screening

Authors: *M. JIANG[1], R. WANG[1], R. SULTANIA[1], E. WHELAN[1], A. TANG[1], T. RATOVISTSKI[1], C. A. ROSS[2];
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Abstract: Huntington's disease (HD) is an autosomal dominant inherited neurodegenerative disease with a combination of motor, cognitive and behavioral impairments caused by an expanded CAG trinucleotide repeat (of variable length) in the huntingtin gene. To develop a more facile cell model, Induced Pluripotent Stem cell (iPSCs) derived from HD patient were immortalized and differentiated into highly homogeneous medium-spiny-neuron like cultures. These immortalized striatal precursor neurons (ISPNs) recapitulated HD-like phenotypes of the parental iPSCs including MAP2/DARPP32 positive immune staining (Akimov et al, 2021). Moreover, HD-ISPNs (180-CAG repeats) displayed greater mitochondrial depolarization and fragmentation than those of control ISPNs (33-CAG repeats). To develop a drug screening cellular platform, HD-ISPNs were placed in a 96-well plate format using CellTiter-Glo luminescent cell viability assay to measure ATP levels after BDNF withdrawal. There were significantly higher levels of toxicity in HD-ISPN lines compared with normal control lines after BDNF withdrawal. Replacement of BDNF (40-50 ng/ml) attenuated mutant HD-induced toxicity in both HD precursor and mature ISPNs. Earlier, we found that huntingtin (HTT) can interact with several protein arginine methyltransferases (PRMTs), and that arginine methylation of endogenous substrates is impaired in HD models (Ratovitski et al, 2015). To evaluate protein arginine methyltransferase (PRMT) as new potential therapeutic target for HD we tested if alterations of PRMT levels affects neuronal survival in our newly generated ISPN HD model. Overexpression of PRMT4, PRMT6, or PRMT8 showed partial protection against growth factor withdrawal-induces stress in ISPNs, whereas knockdown of PRMT6 enhanced mHTT toxicity. Concurrently, a specific PRMT4 inhibitor selectively exacerbated mHTT toxicity in 180CAG line, but not in 33CAG control ISPNs. Our data suggest that modulation of PRMT levels affects survival of HD ISPNs, and further validation of the role of arginine methylation in HD pathogenesis is under investigation. Moreover, we are performing screening tests using libraries of kinase inhibitors and diverse chemical compounds. Taken together, the ISPNs derived from HD iPSCs is a useful cellular model platform for drug screening to combat HD.

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Poster

P277. Huntington's Disease: Models

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Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P277.09

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Next generation reprogramming of human iPSCs to generate disease-specific glutamatergic neurons carrying HTT gene for Huntington's disease research and drug discovery

Authors: T. OOSTERVEEN[1], O. DOVEY[1], S. SALIC[2], M. GAMPERL[2], T. BURCKSTUMMER[2], A. VASILYEV[2], K. FIRTH[1], S. POKORNY[1], A. SIORENTAS[1], A. CONSTANTINE[1], T. FROLOV[1], F. PATELL-SOCHA[1], T. MOREAU[1], M. BROWN[3], M. STEBBEDS[3], J. ANTON[3], M. CONNING-ROWLAND[3], *D. F. FISCHER[3], **M. IOVINO**[3], M. KOTTER[1];
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Abstract: Currently no treatment is available to slow, stop, or reverse the course of Huntington's disease (HD), neurodegenerative disorder caused by a CAG expansion. Physiologically relevant and scalable models of HD are needed to improve research outcomes and drug discovery efficiency. A robust source of human iPSC-derived neurons would offer disease-relevant in vitro model for research and high content drug screens, which could reduce costs, improve screen specificity, and accelerate drug development. Widespread use of human-induced, pluripotent stem cell (hiPSC)-derived, mature cell types however, is restricted by complex and lengthy directed differentiation protocols or cellular reprogramming methods lacking consistency. The novel opti-ox* cellular reprogramming technology, overcomes these restrictions by enabling the precisely controlled expression of transcription factors, and as a result, deterministic induction of a new cell identity. bit.bio ioGlutamatergic Neurons have been generated by NGN2-driven opti-ox reprogramming, which rapidly differentiate into consistent, functional and homogenous cultures of glutamatergic neurons (>80% VGLUT1/2-positive) with electrophysiological activity and 4R MAPT isoform expression after 2 weeks in culture.
Using CRISPR/Cas9-mediated genetic engineering, we have developed opti-ox based isogenic ioGlutamatergic Neurons models carrying heterozygous high CAG repeats in the HTT gene (>50 or >70 repeats), to study Huntington's Disease biology, allow drug screening and functional genomics.
We have performed immunocytochemistry (ICC) on the wild type and HD disease variant ioGlutamatergic Neurons using high content imaging, using pan neuronal (MAP2, TUBB3) and glutamatergic markers (VGLUT1 and VGLUT2) at different time of neuronal differentiation (up to 2 weeks post-revival). In addition, an HTRF® assay was performed in 384-well plate format for the detection of mutant and total HTT protein. CellTiter-Glo® (CTG) assay was performed to measure toxicity after compound treatment. Results demonstrate the suitability of ioGlutamatergic Neurons HTT 50CAG/WT and ioGlutamatergic Neurons HTT 70CAG/WT for high throughput screening.
Together with the wild type ioGlutamatergic Neurons isogenic control, these novel HD cell models offer new avenues into HD drug discovery, to accelerate research and the development of new therapeutics.
* Pawlowski M, et al. Stem Cell Reports. 8(4), 803-812, 2017.

Disclosures: T. Oosterveen: A. Employment/Salary (full or part-time):; bit.bio. **O. Dovey:** A. Employment/Salary (full or part-time):; bit.bio. **S. Salic:** A. Employment/Salary (full or part-time):; bit.bio. **M. Gamperl:** A. Employment/Salary (full or part-time):; bit.bio. **T.**

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Poster

P382. Pain: Nociceptors: Models and Mechanisms

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P382.01

Topic: D.03. Somatosensation – Pain

Support: NIH R01NS111553

Title: Drg increased fto is required for development of neuropathic pain

Authors: *B. WANG;
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Abstract: DRG increased FTO is required for development of neuropathic PainBing Wang, Yize Li, Xinying Guo, Linlin Sun, Jifang Xiao, Shibin Du, Shaogen Wu, Daniel Denis, and Yuan-Xiang TaoDepartment of Anesthesiology, New Jersey Medical School, Rutgers, The State University of New Jersey, Newark, NJ07103, USA; Nerve injury-induced change in gene expression in primary sensory neurons of dorsal root ganglion (DRG) is critical for neuropathic pain genesis. N6-methyladenosine (m6A) modification of RNA represents an additional layer of gene regulation. m^6A is installed by a multi-subunit methyltransferase complex including the methyltransferase-like 3 and 14 (METTL3 and METTL14) and Wilms' tumor 1-associating protein (WTAP) and erased by at least two specific demethylases, fat-mass and obesity-associated proteins (FTO) and AlkB homolog 5 (ALKBH5). Our study showed that peripheral nerve injury increased the expression of FTO, but not METTL3, METTL4, WTTAP and AlkB in injured DRG. This increase occurred exclusively in DRG neurons. No change in expression of DRG FTO was seen after injection of complete Freund's adjuvant. Blocking the nerve injury-

induced increase in DRG FTO alleviated mechanical allodynia and thermal/cold hyperalgesia on days 3, 5, and 7 post-surgery. Mimicking this increase led to the enhanced responses to mechanical, thermal and cold stimuli in naive mice. Our findings indicate that FTO may participate in neuropathic pain development.This work was supported by NIH R01NS111553.

Disclosures: B. Wang: None.

Poster

P382. Pain: Nociceptors: Models and Mechanisms

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P382.02

Topic: D.03. Somatosensation – Pain

Support: NIH R01NS111553

Title: N^6-methyladenosine demethylase FTO stabilizes G9a expression in DRG after peripheral nerve injury

Authors: Y. LI[1], X. GUO[1], L. SUN[1], J. XIAO[1], S. WU[1], B. WANG[1], X. FENG[1], D. DENIS[1], ***Y.-X. TAO**[2];
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Abstract: Although neuropathic pain has been intensively investigated for several decades, how nerve injury produces nociceptive hypersensitivity is still incompletely understood. The evidence has demonstrated that changes in pain-associated gene expression in dorsal root ganglion (DRG) are critical for neuropathic pain. N^6-methyladenosine (m^6A) modification of RNA represents an additional layer of regulation of gene expression. Our pilot data indicated that nerve injury-induced increase in the expression of the m^6A demethylase fat-mass and obesity-associated proteins (FTO) in the injured DRG contributed to neuropathic pain genesis. However, the mechanism underlying this phenomenon is still unclear. We here showed that blocking this increase reversed a loss of m^6A sites in *Ehmt2* mRNA, destabilizing the nerve injury-induced increases in *Ehmt2* mRNA-coding the histone methyltransferase G9a and rescued G9a-controled opioid receptor downregulation. Mimicking nerve injury-induced DRG FTO increase erased m^6A in *Ehmt2* mRNA, elevated the level of G9a and reduced opioid receptor expression in injured DRG. FTO contributes to neuropathic pain likely through stabilizing nerve injury-induced upregulation of G9a, a neuropathic pain in initiator, in primary sensory neurons.

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Poster

P382. Pain: Nociceptors: Models and Mechanisms

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P382.03

Topic: D.03. Somatosensation – Pain

Support: Ro1NS105715

Title: The role of Schwann cells in the onset of neuropathic pain in neurofibromatosis-1

Authors: *N. RAUT[1], L. MAILE[2], I. MITXELENA[2], L. QUEME[3], A. ADLAKHA[2], L. OSWALT[2], D. JOSHI[2], T. RIZVI[4], N. RATNER[5], M. JANKOWSKI[3];
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Abstract: Neurofibromatosis type 1 (NF1) is a genetic disorder that predisposes individuals to benign tumors in the peripheral nervous system (PNS). NF1 patients display multiple clinical manifestations, (e.g., optic gliomas, cutaneous and plexiform neurofibromas and café au lait spots); however, neuropathic pain is one of the primary causes of decreased quality of life in NF1 patients. Specific loss of *Nf1* in SCs and SC precursors has been found to be responsible for neurofibroma formation in a genetically engineered mouse model, but the role of SCs in nociception and pain development in NF1 is not known. In the current study, we wanted to determine whether Schwann cells (SCs), sensory neurons, or both contributed to neuropathic pain-like behaviors in an animal model of NF1. With experimenters blinded to the conditions, we found that in male and female mice, *Nf1* deletion in SCs (Dhh;Nf1f/f) produced mechanical hypersensitivity while *Nf1* deletion in sensory neurons (Pirt;Nf1+/f) did not affect mechanical responsiveness. This was only observed when specifically performing non-evoked behavioral assessments (conflict avoidance). Behavioral hypersensitivity in the SC specific *Nf1* mutants, correlated with sensitization of myelinated A-fiber nociceptors and unmyelinated polymodal C-fibers (CPM) to mechanical stimuli compared to controls using an *ex vivo* skin/nerve/dorsal root ganglion (DRG)/spinal cord preparation. Electron microscopic analysis showed disruption of Remak bundles in SC specific *Nf1* mutants whereas no changes were found in the sensory neuron specific Nf1 mutants. Using transgenic animals with a GqDREADD receptor expressed specifically in SCs (Dhh;Chrm3), we found that chemo-genetic manipulation of SC calcium activity was able to effectively induce mechanical hypersensitivity at the behavioral level and produce sensitization of CPM neurons to mechanical stimuli compared to CNO treated controls. The observed peripheral sensitization correlated with an elevation of numerous cytokines and growth factors in the L2/L3 DRGs as assessed with realtime PCR. Together this suggests that alterations in SCs are directly responsible for changes in specific sensory neuron subtypes that modulate mechanical responsiveness and possibly neuropathic pain development in NF1. This

study has begun to elucidate the specific role of SCs and sensory neurons in the neuropathic pain and may help to identify novel cell-specific treatment strategies to ameliorate pain in NF1 patients.Funding: Grants to MPJ from the NIH (R01NS105715) and Cincinnati Children's Research Foundation.

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Poster

P382. Pain: Nociceptors: Models and Mechanisms

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Program #/Poster #: P382.04

Topic: D.03. Somatosensation – Pain

Support: Anesthesia Innovation and Pilot (AIP) Program at Cincinnati Children's Hospital (LFQ)
NIH grant R01NS113965 (MPJ)
NIH grant R01NS105715 (MPJ)

Title: Loss of enrichment induces widespread muscle mechanical hypersensitivity

Authors: *L. F. QUEME[1,2,3], A. BUTTERFIELD[1], M. C. HOFMANN[1], M. P. JANKOWSKI[1,2,3,4];
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Abstract: The development of chronic musculoskeletal pain has been frequently associated with the presence of high levels of psychological stress. Multiple animal studies have previously shown that a variety of stress paradigms are effective at inducing deep tissue hyperalgesia and enhanced behavioral responses to subthreshold stimuli and injury. Nevertheless, the mechanisms behind stress-induced mechanical hyperalgesia are not clearly understood. Many of the current paradigms to induce stress in rodents fall into the common pitfall that they often utilize physical stimuli that are known to induce changes in sensory function, such as repeated cold exposure or electrical shock. Using non-invasive strategies to induce stress in animal models may uncover new mechanisms that drive stress related hyperalgesia. To address this issue, our lab has adapted a stress paradigm that utilizes environmental enhancement and subsequent loss of enrichment (LOE) to induce stress in mice. Enrichment consisted of 12-16 mice housed together for 3 weeks in a large cage with enrichment materials (e.g., increased bedding). Due to these housing requirements, experiments were performed in male mice only. We then paired this stress paradigm with fore limb ischemia/reperfusion injury (I/R) which served as a model of muscle

hypersensitivity. After 3 weeks of enrichment and 1 week of enrichment removal, our animals developed a prolonged immobility time in a forced swimming test, suggesting increased stress/anxiety-like behaviors. This result correlated with increased weight gain, which is another indicator of stress induction. At baseline (1 week after LOE), the mice also developed a bilateral decrease in mechanical withdrawal thresholds to plantar muscle squeezing, indicating stress related hypersensitivity. Immunohistochemical analysis of the plantar muscles showed increased macrophage infiltration one week after LOE. We then evaluated mice with I/R injury induced one week after LOE and found that these mice displayed a prolonged recovery time (up to 4 additional days to return to baseline levels), compared to normal housing controls that usually recover in approximately 3 days. All data were acquired with experimenters blinded to the various conditions. Our results suggests that LOE can significantly enhance the development of muscle pain after I/R. Together with the immunocytochemical data, results suggest that macrophages could play a role in the development of pain after LOE. These findings highlight the importance of stress in the development of long-lasting pain and may shed light in potential mechanisms behind chronic widespread muscle pain conditions like fibromyalgia.

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Poster

P382. Pain: Nociceptors: Models and Mechanisms

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P382.05

Topic: D.03. Somatosensation – Pain

Support: NIH NINDS R01NS104436
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Title: Altered nociception in a rabbit model of cerebral palsy

Authors: E. J. REEDICH[1,2], C. F. CAVARSAN[1,2], L. T. GENRY[1,2,3], D. BOUDREAU[1,2], M. C. BRENNAN[1,2], A. M. GARRETT[1,2,4], E. MENA AVILA[1,2], L. DOWALIBY[1,2], M. R. DETLOFF[5], *K. A. QUINLAN[1,2];
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Abstract: Cerebral palsy (CP) is the most prevalent motor disability in children, affecting 1:500 live births. CP is caused by injury to the developing fetal and infant central nervous system. The most common comorbidity associated with CP is pain, which is pervasively viewed as a

secondary effect of musculoskeletal abnormalities characteristic of the condition, such as spasticity, hip subluxation and joint contractures. Causal factors linked to CP include hypoxia-ischemia (HI), which could affect both motor and sensory systems. The effect of prenatal HI injury on nociception is entirely unexplored. We hypothesize that prenatal HI injury does not only affect parts of the nervous system involved in motor control, but also directly affects nociceptive sensory neurons, increasing their activity. Here, we investigate the link between prenatal HI injury and nociception in a rabbit model of CP. We evaluate nociceptive behavior in neonatal rabbits after intrauterine hypoxia and use immunohistochemistry to identify anatomical changes to spinal circuitry. We observe that HI-subjected rabbits exhibit mechanical allodynia, even in the absence of motor deficits. These findings indicate that developing sensory circuits may be *more* vulnerable than motor circuits to perturbation by prenatal injury, and that individuals with CP may experience pain as a result of alterations in nociceptive sensory neurons.

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Poster

P382. Pain: Nociceptors: Models and Mechanisms

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P382.06

Topic: D.03. Somatosensation – Pain

Title: Hyperpolarization of the VGluT2 neurons of spinal cord and dorsal root ganglion in mice

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Abstract: Hyperpolarization of the VGluT2 neurons of spinal cord and dorsal rootganglion in mice Pain is an adaptive mechanism; it permits the detection of noxious stimuli throughthe sensory afferents, namely Aδ or C fibers. Some of this fibers colocalize with theVesicular of Glutamate Transporters (VGluTs), Aβ fibers colocalize with VGluT1,meanwhile C fibers colocalize with VGluT2 neurons, it is known that VGluT2 playsan important role in the physiopathology of pain, because its expression increasesin spinal cord after nerve injury. At presynaptic level, deletion of the gene thatcodificates for VGluT2 is related to a diminish in allodynia and hyperalgesia inducedby mechanical or thermal stimuli, without alterations of cold

hypersensitivity inexperimental models of neuropathic pain. **Hypothesis**: The transdermal activationof Arch in VGluT2 neurons of the DRG and their terminals will diminish painperception in a neuropathic pain model. **Objective:** The aim of this study was tocharacterize a subpopulation of the VGluT2 neurons in the DRG and to decreaseVGlut2 excitability in a chronic pain model. **Method:** VGluT2::Cre mice were sexuallymated with Ai35 transgenic mice (VGluT2-Ai35) of Arch. VGluT2-Ai35 and VGluT2hetecygotes (VGluT2 HET) mice with free access to water and food were used in achronic constriction injury (CCI) model of neuropathic pain. After the CCI, and beforesuturing the muscle and skin, 500 nl of virus were administered on the sciatic nerve.Fourteen days after the surgery, nocifensive responses were evaluated using thevon Frey test. Twenty one days after the viral transfection, both the VGluT2 HETand VGluT-Ai35 (constitutive strategy) were evaluated in a CPP model with lightstimulation. Briefly, in the CPP model we stimulate nociceptive fibers using a yellowLED matrix (560 nm). During the first 15 min of the recording (LIGHT OFF) therewas measured the time spent in every compartment of the acrylic box. After thisperiod, there was measured the time spent in the non-illuminated versus theilluminated compartment (LIGHT ON) to activate Arch in the C fibers. **Results:** Thevon Frey test showed that CCI group developed allodynia and hyperalgesia, and thiscondition can be reversed by the light stimulation of the nociceptive fibers usingyellow light in a CPP model in mice that expressed constitutively Arch and thosewere virally transfected. **Conclusions:** Light activation of Arch on VGluT2 neuronscan reduce the pain induced by the CCI and generates CPP in mice.

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Poster

P382. Pain: Nociceptors: Models and Mechanisms

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Program #/Poster #: P382.07

Topic: D.03. Somatosensation – Pain

Support: Wellcome Trust 212302/Z/18/Z

Title: Spike propagation through the dorsal root ganglion investigated using the modified working heart brainstem preparation

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Abstract: Peripheral nerves convey versatile information about the body's environment to the brain, which is interpreted as haptic, somatic and visceral experiences, including pain. Healthy nerves conduct action potentials from their peripheral endings to the spinal cord, where synaptic transmission first takes place. The 'Gate Control' theory assumes that the peripheral somatosensory signals are first integrated in the spinal cord and subsequently analysed in higher brain centres. Here we show that the dorsal root ganglia (DRG) are able to modulate nociceptive signals before they enter the CNS. Simultaneous whole nerve recordings were obtained from the C8 dorsal root (DR) and spinal nerve (SN) in the decerebrate working heart brainstem preparation of the rat (18 day, Wistar rats of either sex). These nerves innervate the median nerve supplying the forepaw. C8 ventral roots were transected. Using in-house custom written scripts to enable spike sorting we were able to identify action potentials in the SN and match these to spikes propagated to the DR. Direct application of GABA (200 μM) into the DRG did not result in any changes in tonic activity in either aspect of the nerve. Noxious stimuli (Randall Selitto) applied directly to the forepaw of the rat resulted in increased firing in both, SN and DR. DRG application of GABA significantly reduced firing frequency in the DR but not in the SN, an effect we are interpreting as GABA-induced filtering of the conduction through the DRG. Spike sorting revealed that the extracellular action potentials traveling along C fibers were more likely to be filtered, in comparison to those in A-type fibers, suggesting that the DRG is able to selectively filter painful stimuli entering the spinal cord via GABAergic mechanisms. This notion was further confirmed in experiments with innocuous stimulation (cotton bud strokes, air puff) and proprioceptive stimulation. These stimuli also resulted in increased firing in both, SN and DR, however, direct application of GABA into the DRG during these circumstances was not able to reduce DR firing rate in the same manner seen with noxious stimulation. Taken together our findings indicate that peripheral somatosensory ganglia may represent a filter or even a 'gate' within the somatosensory system and may serve as a novel therapeutic target for pain relief. As DRGs are not well protected by the blood-brain or blood-nerve barriers, this offers a unique opportunity to target the DRG with BBB-impermeable drugs thereby reducing central nervous system related side effects.

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Poster

P489. Spatial and Feature-Based Attention

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Topic: D.07. Vision

Support: 2R01EY017699
2R01MH064043

P50MH109429

Title: Neural mechanisms of exogenous and endogenous spatial attention in extrastriate visual cortex

Authors: *M. K. ERADATH[1], I. C. FIEBELKORN[3], S. KASTNER[1,2];
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Abstract: Distinct mechanisms of spatial attention have been described based on whether the attention is directed to a spatial location by a salient external cue (exogenous attention) or by an internally generated signal (endogenous attention). Exogenous attention has been described as a bottom-up process, while endogenous attention has been associated with top-down mechanisms. In macaque extrastriate cortex (e.g. area V4), when exogenous cues are presented within a neuron's receptive field (RF), neuronal responses persist beyond the initial sensory response and are thus attention-related. However, it is not known whether this sustained attentional response in V4 can be initiated and maintained in the absence of an exogenous cue and also occurs in response to an endogenous cue. Here, we used a spatial attention task (Egly - Driver task), in which monkeys detected a near threshold contrast change by directing covert attention to a spatial location based on either a visual cue at the target location (exogeneous allocation of attention) or a symbolic cue (color change) at central fixation (endogenous allocation of attention). The symbolic cue (i.e., the endogenous cue) should not drive any sensory-evoked response, given that the cue is not presented in the RF of the V4 neuron. The target appeared after a variable delay period in a neuron's RF. We performed simultaneous multi-contact recordings from area V4 and the lateral intraparietal area (LIP). Significant delay period activity was observed in all V4 layers both for exogenous and endogenous conditions. In V4, neural responses evoked by exogenous cues were first observed in granular layers (51ms) followed by infra- and supragranular layers (57ms and 54ms, respectively). The endogenous cues on the other hand, evoked responses first in supragranular layers (53ms) of V4 followed by infra- and granular layers (60ms and 58ms, respectively). In LIP, the evoked response latency for exogenous cues was shorter in deeper layers (53ms) compared to superficial layers (56ms) while it was shorter in superficial layers (48ms) compared to deeper layers (58ms) for endogenous cues. Our results are consistent with bottom-up activation of attention-related delay activity in response to exogenous cues and top-down activation of attention-related delay activity in response to endogenous cues. Furthermore, the present findings provide evidence of different neural mechanisms leading to attention-related neural responses in extrastriate visual cortex, depending on whether attention is deployed in response to either an exogenous or an endogenous cue.

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Poster

P489. Spatial and Feature-Based Attention

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Topic: D.07. Vision

Support: NIH Grant EY018875

Title: Functional specificity of attentional modulation in the disparity domain

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Abstract: It has been suggested that absolute disparity cues, unlike relative disparity cues, are perceptually inaccessible (Chopin et al., JVis, 2016). If so, processing of absolute disparity information should be less affected by manipulation of attention. Steady-state visual evoked potentials were recorded (N=20) in response to dynamic random dot stereograms (DRDS) alternating at 2 Hz between a flat plane at zero disparity and a 0.5 cpd crossed-disparity sine-wave grating. Disparity was measured at 10 equal log steps. In one condition, participants attended to monocular nonius lines at fixation and pressed a button when a color change occurred. In a free attend condition, participants attended to the DRDS stimulus while maintaining fixation with no button presses. Evoked responses were robust at the first four harmonics of the stimulus frequency (1F, 2F, 3F, 4F). The disparity response function at 1F in the free attend condition was leftward shifted relative to the attend nonius condition, consistent with an input-gain mechanism. The 3F and 4F responses showed no effect of attention. We hypothesized that the unmodulated 3F and 4F signals came from the leading edge of the response and conducted a follow-up experiment to determine the timing of the task effect and the relative timings of absolute and relative disparity encoding (N=9). The disparity grating response (relative disparity) started at around 100 ms and attention began to modulate the response around 50 ms later. Responses to a disparity plane (absolute disparity) and grating diverged at the same ~150 ms latency. Importantly, the task had no effect on the response to the absolute disparity condition at any latency. Our results suggest that the earliest disparity responses represent the encoding of absolute disparity and that responses to absolute disparity are unaffected by attention, whilst responses to relative disparity, once they have been extracted, are modulated by attention.

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Poster

P489. Spatial and Feature-Based Attention

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Topic: D.07. Vision

Support: NIH NEI R00 EY018683
Hitchcock Foundation Pilot Grant to F.B.
NIH NEI F32 EY023165 to J.R.H-S.

Title: Attentional modulation of spike count correlations among pairs of anatomically connected V1 neurons

Authors: *S. SHAH[1], B. CARR[1], J. R. HEMBROOK-SHORT[2], V. L. MOCK[2], F. BRIGGS[1,3]; [1]Neurosci., Univ. of Rochester Med. Ctr., Rochester, NY; [2]Physiol. & Neurobio. Dept., Geisel Sch. of Med. at Dartmouth, Lebanon, NH; [3]Brain and Cognitive Sci., Univ. of Rochester, Rochester, NY

Abstract: Visual attention augments behaviorally relevant visual stimuli by modulating several aspects of neuronal activity among visual neurons, including altering firing rates, variability, and pairwise spike count correlations. Whereas attention was initially shown to reduce spike count correlations among pairs of simultaneously recorded visual cortical neurons, more recent evidence suggests that the effect of attention on pairwise spike count correlations varies dependent on cell type, laminar location, and whether neurons are located within the same visual area or spanning different visual areas. One factor that has not been considered is actual anatomical connectivity. i.e., does attentional modulation of spike count correlations among pairs of visual cortical neurons depend on whether or not they are connected? Additionally, many attention effects also depend on whether neurons encode task-relevant feature information. Here we examine the effect of attention on spike count correlations with respect to both anatomical connectivity and task-relevant feature selectivity of neuronal pairings. Specifically, we hypothesize that attention will reduce spike count correlations for neuronal pairs that are anatomically connected and tuned for task-relevant features. Furthermore, we predict that attention will not affect spike count correlations or even increase spike count correlations for neuronal pairings that are tuned for orthogonal features and/or are unconnected. To test this, we utilized multielectrode arrays inserted into V1 of macaque monkeys to simultaneously record from multiple neurons while monkeys performed an attention task. The attention task involved multiple visual discriminations of stimulus contrast, orientation, or color, all requiring shifts in covert visual spatial attention. We also measured neuronal tuning for visual stimulus features independent of the attention task. Preliminary data suggest that attention reduces spike count correlations among monosynaptically connected pairs of V1 neurons. However, attentional modulation of spike count correlations among unconnected pairings of V1 neurons was not significant. These data suggest that attentional modulation of correlated neuronal activity depends on actual connectivity.

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Poster

P489. Spatial and Feature-Based Attention

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Topic: D.07. Vision

Support: DFG Grant 331514942

Title: Tracking visual stimulus information across cortical layers in macaque's area V1 for identification of neuronal gating mechanisms

Authors: E. DREBITZ, L.-P. RAUSCH, E. DOMINGO GIL, R. BARTELS, *A. K. KREITER; Brain Res. Inst., Univ. of Bremen, Bremen, Germany

Abstract: Successful interaction with and within our environment requires the brain to deal with a large amount of visual information at once. Often that information represents multiple independent objects that are close together or even overlap. Therefore, the highly convergent neuronal connections along the visual processing pathways often provide inputs representing multiple separate objects to neurons located downstream. Nevertheless, these neurons respond as if only the currently relevant stimulus is present. The Routing by Synchrony hypothesis explains this selective processing by a selective synchronization of neuronal activity between neurons encoding relevant information. It has been shown that receiving neurons synchronize their activity with those input neurons representing the relevant stimulus within the gamma-band (30 - 100 Hz) while exhibiting almost no coherence with input neurons representing an irrelevant stimulus.Here we investigate whether a similar mechanism of dynamic information routing also exists between different layers of V1. Moreover, we tested whether effective information transfer between neurons in different cortical layers depends not only on the coherence of oscillatory activity but also on a particular instantaneous phase relation between sender and receiver neurons. These two aspects of synchronization (coherence, phase relation) are critical elements of a Routing by Synchrony mechanism.To this end, we recorded neuronal activity in two macaque monkeys (*Macaca mulatta*) striate cortex (V1). Neuronal recordings were conducted with linear multi-contact probes (100 µm spacing) while the animals performed a shape tracking task. On top, each visual stimulus changed semi-randomly in luminance, resulting in a stimulus-specific tagging of evoked neuronal activity. We calculated the spectral coherence between the current-source density (CSD) at different cortical layers and the time- and frequency-varying stimulus tags to quantify the stimulus information transfer. We found strong gamma-band coherence between neurons of different layers with comparably slight differences between conditions in which the processed stimulus was relevant vs. irrelevant. However, the transmission of stimulus-specific information depended on the instantaneous phase difference between the otherwise identical gamma-band oscillations of the neurons in granular and supragranular layers. In summary, our results deliver strong evidence for the proposed Routing by Synchrony mechanism by confirming that both coherence and specific phase relations determine the effectiveness of information transfer within a cortical column in V1.

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Poster

P489. Spatial and Feature-Based Attention

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Topic: D.07. Vision

Support: ONR Grant N000141712480
Materials Research Science and Engineering Centers (MRSEC) at University of Chicago

Title: A machine vision approach to quantifying the effectiveness of cephalopod camouflage in natural scenes

Authors: *A. RISBUD[1], S. WANG[1], N. GOSWAMI[3], L. C. OSBORNE[4], K. BURESCH[5], C. CHUBB[6], R. HANLON[5], S. E. PALMER[2];
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Abstract: Rapid adaptive camouflage is a critical defense mechanism for cephalopods. The characterization of cephalopod camouflage has thus far been restricted to qualitative descriptions or quantitative measures that rely only on a single low-dimensional factor, such as spatial frequency or color. While useful, these characterizations do not capture the multi-faceted nature of camouflage, nor do they provide a comprehensive metric by which the degree and effectiveness of cephalopod camouflage from the perspective of a given predator can be quantitatively measured in a given scene. Here, we propose a metric that integrates lower- and higher-dimensional visual features to read out the degree of texture matching in an image of a camouflaged cephalopod on a background texture. This metric is motivated by an algorithm in machine vision that decomposes an input image into its constituent artistic “style” and “content” components, using a cascade feedforward artificial neural network (VGG-19). The aforementioned integration of lower- and higher-dimensional visual features is made possible by the bank of visual filters of varying spatial scales present in the VGG-19 architecture. This next texture-distance metric will allow researchers to quantify camouflage in a variety of scenes, and possibly develop and test new camouflage designs based on these metrics. Pairing this metric with the results of high-resolution eye-tracking data might also allow researchers to better understand potential targets of visual fixation in natural scenes.

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Poster

P580. Sensory Motor Systems II

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Topic: F.01. Neuroethology

Support: PAPPIT IN306918
PAPIME PE306318

Title: Ethogram of the Mexican four-eyed octopus: Octopus maya.

Authors: *D. A. GONZÁLEZ-NAVARRETE[1], F. VERGARA-OVALLE[2], P. GARCÍA-ANDALUZ[2], F. AYALA-GUERRERO[2], D. B. PAZ-TREJO[3], H. SANCHEZ-CASTILLO[4]; [1]Univ. Nacional Autónoma De México, Ciudad de México, Mexico; [2]Univ. Nacional Autónoma de México, Ciudad de México, Mexico; [3]Univ. Nacional Autonoma de Mexico, Mexico City, Mexico; [4]Univ. Nacional Autonoma De Mexico. Fac Psicologia, Mexico City, Mexico

Abstract: Historically in neuroscience, behavioral studies have been focused mainly in vertebrates, however, research on invertebrate behavior has been increasing because the potential for research that these models presents. Nowadays, when we talk about mollusk behavior, cephalopods are the first group that stand out, however, there is a lack of research with the endemic species from Mexico, particularly with *Octopus maya*. This octopus has shown to adapt well to laboratory conditions. Additionally, the breeding in captivity has been successfully achieved through several generations, giving it a great advantage as a research model. These characteristics, could reduce the number of variables that can affect the behavioral studies. In this way, with the aim of knowing the behaviors that this species can perform in controlled conditions and work properly with in the laboratory, the main objective of this study was carry out an ethogram that describes the behavioral repertoire that *O. maya* displays in controlled conditions. Individuals of *O. maya* (6-20g) were used and maintained in tanks with a closed circulation seawater system and illuminated with a red light of 30 lx in a 12:12 LD cycle. Nine of this individuals were used to an *ad libitum* sampling of behavioral repertoire to name, categorize and define operationally the recorded behaviors. Another four individuals were used to establish the day/night activity patterns, (length and frequency of the behaviors along the day). The obtained results showed that *O. maya* has a behavioral repertoire with eight postures and twenty behaviors, which we included in six behavioral different categories. Also, this species shows a differentiated activity patterns between day and night, with a peaks of activity, more distribution and frequencies of activity behaviors mainly in the light hours. This results showed that *O. maya* presents behaviors comparable with other octopus's species and support that it is an organism

that can be proposed as a research model. Also, knowing the behavioral repertoire of this species allows greater control in subsequent investigations related to behavior and provide a great tool to constantly monitor the health status of organisms and its use for research, being a precedent for future behavioral research in this species related to neuroscience and cognition.

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Poster

P580. Sensory Motor Systems II

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Topic: F.01. Neuroethology

Support: Christopher Newport Univ. 2020 Summer Scholars Program
Christopher Newport Univ. 2020 Research Captains Program

Title: Female zebrafish (Danio rerio) Demonstrate Stronger Preference than Males for Established Shoals over Newly-Formed Shoals in the Three-Tank Open-Swim Preference Test

Authors: *I. DANSTROM[1], **C. KOON**[1], K. M. WIENS[2], *A. J. VELKEY, II[1];
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Abstract: Zebrafish (*Danio rerio*) display complex social behaviors such as shoaling and schooling, making them an attractive model for investigating normal social behavior as well as exploring conditions of impaired social function such as autism spectrum disorders. Recognizing group dynamics is an important aspect of successfully navigating social situations, and this capability may be impaired for individuals with autism. Several previous studies have examined the responses of fish in a test shoal with a novel conspecific and vice versa, but no published study has examined the response of a solitary test subject to shoals with different degrees of intra-group familiarity. Newly-formed and established shoals exhibit distinct behavior patterns and inter-member interactions that could convey the group's social stability. We used a three-chamber open-swim preference test to determine whether individual zebrafish show a preference for an established shoal over a newly-formed shoal. Results indicated that both sexes maintained greater proximity to arena zones nearest to the established shoal stimulus. However, there was a sex difference in movement patterns with males spending more time moving across all zones and demonstrating higher levels of behavioral diversity. This novel model using established versus newly-formed shoals can be used in future studies testing transgenics and pharmacological treatments that mimic autism spectrum disorder and other disorders that affect social interaction.

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Poster

P580. Sensory Motor Systems II

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University of Washington Institute for Neural Engineering fellowship funding
Ocean Memory Cognition and Genomics Seed Grant

Title: Strategies of octopus arm control in the absence of visual feedback

Authors: *D. M. SIVITILLI[1], W. WEERTMAN[3], J. ULLMANN[2], J. R. SMITH[2], D. H. GIRE[1];
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Abstract: The octopus coordinates its multiple, highly flexible arms with the support of a complex distributed nervous system. The octopus' suckers, staggered along the length of each arm, are employed in a range of behaviors such as locomotion, exploration, foraging, and manipulation, for which the suckers serve as the primary medium of sensory transduction and as the primary motor effectors. In coordinating limbs with seemingly infinite degrees of freedom across a variety of adaptive behaviors, the octopus appears to have solved a significant control problem facing the field of soft-bodied robotics. To study the strategies that the octopus uses to coordinate its arms and suckers, we designed and 3D printed visually occluded single arm foraging tasks and tracked arm kinematics as the octopus attempted to find and retrieve a food reward. By varying the location of the food item within this task and the features of the task itself, we can characterize how the arms and suckers adapt to their environment. Our results suggest that the octopus relies on a contact-based navigation strategy that emerges from local sucker coordination within an arm to simplify the control of its soft, highly flexible limbs.

Disclosures: D.M. Sivitilli: None. **W. Weertman:** None. **J. Ullmann:** None. **J.R. Smith:** None. **D.H. Gire:** None.

Poster

P580. Sensory Motor Systems II

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P580.04

Topic: F.01. Neuroethology

Support: Brain Research Foundation BRFSG-2019-08 (PI Yttri)

Title: B-soid, an open-source unsupervised algorithm for identification and fast prediction of behaviors.

Authors: *A. HSU, E. A. YTTRI;
Biol. Sci., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Studying naturalistic animal behavior remains a prohibitively difficult objective. Recent machine learning advances have enabled limb localization. Extracting behaviors, however, requires ascertaining the spatiotemporal patterns of these positions. To provide the missing bridge from poses to actions and their kinematics, we developed B-SOiD - an open-source, unsupervised algorithm that automatically identifies behavior without user bias (Nature Communications, 2021). By training a machine classifier on pose pattern statistics clustered using new methods, our approach achieves greatly improved processing speed and the ability to generalize across subjects or labs. By using a frameshift alignment paradigm, B-SOiD overcomes previous temporal resolution barriers that prevent the use of other algorithms with electrophysiological recordings, providing greater resolution for neural dynamics. With this behavioral segmentation paired with electrophysiological recordings, we demonstrate the representation of untrained, naturalistic movements, as well as how the selection of these actions and population dynamics change over time. Using only a single, off-the-shelf camera, B-SOiD provides sub-action categories and kinematics for trained or untrained behaviors in invertebrate, rodent, or primate models. These behavioral and kinematic measures are difficult but critical to obtain, particularly in the study of rodent and other models of pain, OCD, and movement disorders.

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

Poster

P580. Sensory Motor Systems II

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P580.05

Topic: F.01. Neuroethology

Support: NSF CRCNS 1822550
the Vannevar Bush Faculty Award ONR N000142012828

Title: Quantitative analysis of somersaulting in Hydra vulgaris

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

Abstract: Cnidarians are representatives of the first animals to evolve a nervous system, and use their simple nervous system, called a nerve net, to sense the external world, integrate information, and generate selective behaviors. Yet how the nerve net encodes these sensorimotor mechanisms and behaviors remains to be elucidated. In order to understand these mechanisms, it is important to first establish methods to detect and analyze different behaviors quantitatively. To explore this, we have studied the behavior of *Hydra vulgaris*. This fresh-water cnidarian is a small transparent polyp, 0.5-1.5 cm long, with 200 to 6,000 spiking neurons, organized into an ectodermic and an endodermic nerve net, and whose activity can be reconstructed with calcium imaging. We used DeepLabCut, based on deep convolutional networks, to track the position of the animal's body during free behavior. In those data, we were able to detect the *Hydra's* contractions and also somersaulting, acrobatic locomotion in which the animal fully rotates around a horizontal axis with the foot passing over the head. The results showed that contractions could be automatically detected by measuring changes in body length whereas somersaults could be detected by significant changes in the velocity of the basal disc. Furthermore, we found characteristic time scales for each of the five sequential components of somersaulting: (1) body elongation - tentacle attachment (508.6 ± 78.0 sec), (2) foot detachment (3.9 ± 0.8 sec), (3) body contraction (16.5 ± 1.3 sec), (4) foot attachment (19.1 ± 2.7 sec), and (5) tentacle detachment (162.6 ± 18.4 sec). The neural bases of these different components are being investigated. Characterizing quantitative behavior in primitive nervous systems is a first step toward understanding the neural code that relates neuronal activity to behavior, a code whose basic strategies may be conserved in other cnidarians and bilaterians.

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

Poster

P580. Sensory Motor Systems II

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Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P580.06

Topic: F.01. Neuroethology

Support: NIH Grant UF1NS115817

Title: Molecular study of the structure and function of the octopus sucker ganglion

Authors: C. S. OLSON[1], N. G. SCHULZ[2], *C. W. RAGSDALE[3];
[1]Univ. of Chicago Committee On Computat. Neurosci., Chicago, IL; [2]Univ. of Chicago

Committee On Development, Regenerative and Stem Cell Biol., Chicago, IL; [3]Univ. of Chicago Committee On Neurobio., Chicago, IL

Abstract: Each sucker on the octopus arm has a dedicated ganglion positioned between the axial nerve cord, which runs down the center of the arm, and the base of the sucker. The functional purpose of these sucker ganglia remains obscure: some authors claim a primary motor function (Guérin, 1908), some argue for a mainly sensory role (Rowell, 1963), while Graziadei (1965) proposed a combination of the two. We examined the structural organization and molecular composition of the sucker ganglion in adult octopus arms (*Octopus bimaculoides*, n=5) with molecular methods including immunohistochemistry for acetylated alpha-tubulin, phalloidin and DAPI labelling, and in situ hybridization for pan-neuronal and neuronal subtype markers. The sucker ganglion is ellipsoid in shape and anchored in the stalk of the sucker by the acetabulo-brachial musculature. As demonstrated by acetylated alpha-tubulin immunostaining and pan-neuronal marker gene expression (*CPLX1*, *MUNC18*, *SYT1*), the sucker ganglion has an unusual organization for an invertebrate ganglion. The neuropil of the ganglion is dense aboral to the sucker, with neuronal cell bodies concentrated on the sucker (or oral) side of the ganglion. With neuronal subtype markers, we found clear districting within the ganglion's cell body layer. Cholinergic neuron markers, *VACHT* and *CHAT*, are localized to the outside edges of the ganglion as well as a region in the center. A potential motor neuron marker, *LHX3*, similarly labels the outside edges along with the aboral portion of the cell body layer. *PIEZO*, a mechanosensory ion channel gene, is detected heterogeneously throughout the ganglion, often forming clumps of cells. These results suggest that the sucker ganglion is composed of both motor and sensory components, and raise the possibility of a differential distribution of sensory and motor neurons within the ganglion.

Disclosures: C.S. Olson: None. **N.G. Schulz:** None. **C.W. Ragsdale:** None.

Poster

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Program #/Poster #: P580.07

Topic: F.01. Neuroethology

Support: NSF Grant DBI2015317

Title: When speed is independent of size: quasi-static mechanics of cyclic Aplysia feeding

Authors: *V. FAN[1], A. SKALSKI DE CAMPOS[1], K. WANG[1], J. P. GILL[1], G. P. SUTTON[2], H. J. CHIEL[1];
[1]Case Western Reserve Univ., Cleveland, OH; [2]Univ. of Lincoln, Lincoln, United Kingdom

Abstract: How do rhythmic behaviors change with size? How can this affect neural control? In animals that primarily use inertial forces to generate rhythmic behaviors, smaller animals generate faster cycle times than larger animals. For example, in humans, children at the age of 5 years have faster step cycle times than adolescents at the age of 19 [Lythgo et al. Gait and Posture 2011]. Biomechanical analysis would predict this result: The resonant frequency of a system is proportional to the stiffness divided by the mass (i.e., $(k/m)^{(1/2)}$). Since stiffness is proportional to length L, and mass is proportional to the volume, L^3, the resonant frequency is proportional to $(L/L^3)^{(1/2)}$ which equals 1/L. In turn, this implies that the resonant frequency will decrease as mass increases, since L is proportional to $m^{(1/3)}$, and thus animals with larger masses will have lower resonant frequencies (i.e., 1/L will be smaller with larger mass). In contrast, when movement is dominated by elastic forces (i.e., when the system is quasi-static), displacement is primarily generated by storing energy in a spring. This scales differently: in these systems, force is proportional to stiffness times displacement (a spring equation), or F = k x. On the left-hand side of this equation, F is equal to the cross-sectional area of the muscle, which scales as L^2 times the muscle activation. On the right-hand side, the stiffness scales with the length of the muscle, and so the product k x scales as L^2. In turn, this predicts that the time to generate a motion will be scale *independent* (assuming the same activation). To test this prediction, we have measured the timing of biting and swallowing in the marine mollusk *Aplysia californica*, which grows over several orders of magnitude in mass over its lifetime. We observed that the durations of bites and swallows were essentially unchanged over a wide range of animal mass, consistent with the prediction of the theory. In contrast, peak forces are predicted to be proportional to the cross-sectional area of the muscle, which will increase with the mass of the animal (i.e., $m^{(2/3)}$). Measurements of the peak forces in *Aplysia* plotted on a log-log scale yielded a slope of approximately 0.7, consistent with the theory's predictions. Since muscles in *Aplysia*, in general, do not spike, muscle innervation must increase in proportion to the muscle's area as a function of the animal's mass. This hypothesis will be tested in future work.

Disclosures: V. Fan: None. **A. Skalski de Campos:** None. **K. Wang:** None. **J.P. Gill:** None. **G.P. Sutton:** None. **H.J. Chiel:** None.

Poster

P588. Neurobiological Bases of Social Behavior

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P588.01

Topic: F.02. Behavioral Neuroendocrinology

Support: NIDA Grant R01DA039062

Title: Astrocytes in the medial amygdala regulate playfulness in real time in juvenile rats

Authors: *J. VANRYZIN, A. E. MARQUARDT, M. M. MCCARTHY;
Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: The medial amygdala (MeA) is the site of masculinization of juvenile social play behavior in rats. We have established that during neonatal development, microglia in the male amygdala phagocytose more newborn astrocytes than in females. By the juvenile age, males have fewer astrocytes in the MeA. The sex difference in MeA astrocyte number is inversely related to both the activation of MeA neurons as well as the amount of play, as males display more frequent and vigorous rough-and-tumble play as juveniles and have more immediate early gene expression following a play bout (VanRyzin et al., Neuron, 2019). Whether the developmental determination of a sex difference in astrocyte number has direct implications on play as it is occurring has been unknown. Astrocytes are potent modulators of neural activity via gliotransmitter signaling, leading us to hypothesize that astrocytes in the MeA regulate rough-and-tumble play by influencing the activity of play-relevant neurons. To test this hypothesis, we selectively modulated these cells using astrocyte-specific DREADD viruses and found that HM3D stimulation of MeA astrocytes decreased juvenile play while HM4D stimulation increased play, in real time. Infusing an adenosine (a known gliotransmitter) receptor 1 (A1R) agonist directly into the MeA via bilateral cannulation similarly and immediately decreased play, suggesting that astrocytes may be regulating neural activity and behavior through this gliotransmitter. To determine the phenotype of play-active cells, we used RNAscope to identify the neurochemical phenotype of cells expressing the immediate early gene, Egr1, following a single play bout. Males had significantly more Egr1+ cells in the MeA than females, and the majority (>80%) of Egr1+ cells were also VGAT+ indicating that the play-active neurons are largely GABAergic. Future studies will determine the precise localization of adenosine receptors within the MeA, and determine the projection profile of play-active GABAergic cells. Together, these data suggest that astrocytes within the juvenile MeA provide real-time modulation of neural activity and play behavior.

Disclosures: J. Vanryzin: None. **A.E. Marquardt:** None. **M.M. McCarthy:** None.

Poster

P588. Neurobiological Bases of Social Behavior

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Program #/Poster #: P588.02

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH grant 5R00NS089824
2017 NARSAD Young Investigator Grant

Title: Lack of pattern decorrelation in the prelimbic circuit disables social discrimination in Mecp2-deficient mice

Authors: *P. XU, Y. YUE, J. SU, X. SUN, H. DU, Z. LIU, R. SIMHA, C. ZENG, H. LU; George Washington Univ., Washington, DC

Abstract: Social interactions involve a range of complex considerations, from awareness of one's internal state to assessment of myriad social cues and social memory. Much of the processing of socially relevant information takes place in the medial prefrontal cortex (mPFC) circuit, which supports cognition, memory, and decision-making. The mPFC responds to social stimuli and carries both social and spatial information, but it is unclear how its circuit activity encodes different stimuli to guide social discrimination. Because dysfunction of the prefrontal cortical circuit has been associated with social deficits demonstrated in Rett syndrome (RTT), which is caused by loss-of-function mutations in the X-linked gene Methyl-CpG-binding protein 2 (*MECP2*), we were able to compare mPFC function in both wild-type and mutant mice to examine circuit differences underlying a well-documented social deficit. Using a miniature microscope, we conducted calcium imaging of prelimbic mPFC pyramidal neurons in 5-month-old female heterozygous *Mecp2*$^{+/-}$ (RTT) mice and wild type (WT) mice while they were engaged in the three-chamber social interaction assay. We found that the mutant excitatory mPFC circuit is less responsive to both social and nonsocial stimuli in RTT mice, which limits its ability to decorrelate the activity patterns encoding different stimuli. Using simultaneous optogenetic manipulation and in vivo calcium imaging, we found that disinhibition of the mPFC excitatory circuit restored pattern decorrelation for social information coding and the social preference of RTT mice. Inhibition or over-activation of WT mPFC excitatory circuit causes a failure of pattern decorrelation in WT mice and caused them to exhibit the same deficit in social discrimination as RTT mice. Normal social behavior therefore depends on pattern decorrelation of mPFC circuit activity and normal E/I balance. Stimulation of the mPFC could serve as a potential strategy to mitigate some of the core symptoms of RTT patients.

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Poster

P588. Neurobiological Bases of Social Behavior

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Program #/Poster #: P588.03

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant F31MH123025
NIH Grant R01DA0396062
NIH Grant R24MH114815

Title: Sex differences in the transcriptional networks underlying playfulness suggest a distinct function for play in males compared to females

Authors: *A. E. MARQUARDT[1], J. W. VANRYZIN[2], S. A. AMENT[3], M. M. MCCARTHY[2]; [1]Program in Neurosci., [2]Dept. of Pharmacol., [3]Inst. for Genome Sci. and Dept. of Psychiatry, Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Social play, or rough-and-tumble play, is a dynamic behavior expressed by most juvenile mammals. While its function is debated, a prevailing hypothesis suggests play serves to sculpt and refine neural circuitry enabling expression of appropriate adult behavior. Importantly, in most species, males play more than females, a sex difference driven by the medial amygdala (MeA). To investigate whether the transcriptional signatures underlying play also differ by sex, we performed RNA-sequencing of MeA samples from high- and low-playing juvenile rats of both sexes. Using Weighted Gene Co-expression Network Analysis (WGCNA), we identified 22 co-expression modules, or networks of genes highly correlated in expression across samples. Of the 12 modules significantly associated with play (for $p<0.05$), almost all (11/12; ~92%) are sex-specific in expression, correlating with expression of play in one sex only. These data suggest there is a distinct transcriptomic profile associated with playfulness in the MeA of males compared to females, a noteworthy finding given the MeA regulates many sex-typical adult social behaviors. We propose that this is no coincidence: play-associated gene networks in the MeA are sex-specific because play modulates circuitry driving different adult behaviors depending on sex. To investigate this, we created perforated Plexiglass cage dividers to separate juvenile cagemates, preventing play while allowing for other forms of social interaction to occur. We hypothesized that preventing play would result in impairments in later-life behavior that differed in males compared to females. Supporting our hypothesis, preventing juvenile play impaired object memory and copulatory behavior and resulted in a hypersocial phenotype only in adult males. Surprisingly, we observed no effects on the same behaviors in females nor on maternal behavior, or on sex preference, social recognition, or anxiety-like behavior in either sex. Future studies will examine whether our identified sex-specific gene modules have a causal role in juvenile playfulness, an idea supported by preliminary siRNA experiments, and/or in later-life behavior, using a viral approach to determine if driving expression of modules of interest in the juvenile MeA can rescue behavioral deficits in animals prevented from playing. Together, these analyses will provide novel insight into the ultimate function of play and how and why this may differ by sex.

Disclosures: A.E. Marquardt: None. **J.W. VanRyzin:** None. **S.A. Ament:** None. **M.M. McCarthy:** None.

Poster

P588. Neurobiological Bases of Social Behavior

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Program #/Poster #: P588.04

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH Grant R01DA039062 to MMM
Simons Foundation pilot award to MMM

Title: Characterization of playfulness and social motivation in juvenile rats in a model of neuropsychiatric developmental disorders

Authors: *S. E. ASHTON[1], S. N. YAVASANI[3], S. PATTANAIK[3], K. E. KIGHT[2], M. M. MCCARTHY[2];
[1]Program in Neurosci., [2]Dept. of Pharmacol., Univ. of Maryland Sch. of Med., Baltimore, MD; [3]Poolesville High Sch., Poolesville, MD

Abstract: Juvenile social play is conserved across mammals, including humans, and is impaired in children with male-biased neuropsychiatric developmental disorders (NDDs) such as autism spectrum disorder, ADHD and possibly schizophrenia. Because play is thought to be important to development of later life social skills, it is plausible that the disrupted play in NDDs contributes to the lasting social impairments common in these disorders. Our lab has recently developed a "two-hit" rat model of NDDs by combining two established risk factors: haploinsufficiency of the candidate gene Nrxn1α (Sprague Dawley-Nrxn1^{tm1sage}) and early life inflammation, as modeled by PolyI:C injection on postnatal days 8 and 10. Interestingly, previous work in the lab has demonstrated that this "two-hit" model results in impaired playfulness in juvenile males but not females. We hypothesize that these deficits arise from reduced motivation for social interaction. We first optimized a novel assay for assessing social motivation in juvenile rats and found no sex differences in the willingness to push through a swinging door for access to a playmate in wildtype animals, even though males play more intensely than females. However, there was a positive correlation between the number of door entries and the number of play behaviors in males but not females, suggesting motivation for social interaction may be more closely related to playfulness in males. Contrary to our prediction, we found no reduction in the number of door entries following PolyI:C treatment in animals haploinsufficient for Nrxn1α compared to vehicle treatment, regardless of sex. This suggests that the impaired playfulness in the "two-hit" males is likely not due to reduced motivation to engage with a peer, but rather may be specific to play itself. To more thoroughly characterize juvenile social behavior in males and females of the "two-hit" model, we have employed DeepLabCut multi-animal pose estimation combined with SimBA classification. This machine learning approach has the potential to reveal subtle differences in the sequence and nature of playfulness that have thus far been difficult to identify by the traditional manual scoring of discrete behaviors. Together, this work provides insights into the origin of the lasting social deficits central to male-biased NDDs.

Disclosures: S.E. Ashton: None. **S.N. Yavasani:** None. **S. Pattanaik:** None. **K.E. Kight:** None. **M.M. McCarthy:** None.

Poster

P588. Neurobiological Bases of Social Behavior

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Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P588.05

Topic: F.02. Behavioral Neuroendocrinology

Support: R01-NS113104-01

Title: The role of pair bonding and oxytocin receptor expression in partner-seeking behavior during loss in male prairie voles

Authors: *E. M. VITALE, S. A. SANCHEZ, L. H. HALE, A. S. SMITH;
Dept. of Pharmacol. & Toxicology, Univ. of Kansas, Lawrence, KS

Abstract: In social species such as humans and the socially monogamous prairie vole, the dissolving of social bonds is disruptive and leads to increased stress responsivity and a strong desire for reunion with the lost individual. Indeed, male prairie voles that lose their partner are more anxious than those that maintain their partnership. Despite being separated from their partner for up to two weeks, males will retain a preference for their partner over an unfamiliar female. This suggests maintenance of partner recognition in the absence of physical contact and cohabitation. The oxytocin (OT) system is critical for the formation of pair bonds, and it is also involved in social recognition and social memory. Thus, long-term changes in the oxytocin system resulting from cohabitation and pair bonding may contribute to partner-seeking behavior. Additionally, if a bond is not robust enough to form the neural framework necessary for retaining social memory, this may inhibit partner-seeking behavior following loss. Here, we hypothesize that pair bonding is associated with OT receptor expression in brain regions which regulate pair bonding and social recognition. We predict that changes in the OT system are maintained after loss of a bonded partner, indicated by a strong partner preference, and preserves social memory to promote partner-seeking behavior. We used a social conditioned place preference test to assess the rewarding properties of the subjects' partner in pair-bonded animals that remained in an intact pair, pair bonded animals that lost their partner, and non-bonded animals that lost their partner. We found that pair bonded animals that lost their partner spent more time in the chamber associated with their partner than pair bonded intact animals and non-bonded animals that lost their companion. We are currently examining OTR binding in several brain regions associated with pair bonding and social recognition, including the prefrontal cortex, nucleus accumbens, lateral septum, amygdala, hippocampus, and ventral tegmental area.

Disclosures: E.M. Vitale: None. **S.A. Sanchez:** None. **L.H. Hale:** None. **A.S. Smith:** None.

Poster

P588. Neurobiological Bases of Social Behavior

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Program #/Poster #: P588.06

Topic: F.02. Behavioral Neuroendocrinology

Support: NIH 3R01NS113104

Title: Bed nucleus of the stria terminalis corticotropin-releasing hormone and nucleus accumbens dopamine modulation of pair bonding in stress-naïve and socially defeated prairie voles (Microtus ochrogaster)

Authors: *M. TICKERHOOF, A. S. SMITH;
Dept. of Pharmacol. & Toxicology, Univ. of Kansas, Lawrence, KS

Abstract: Social relationships and affiliation can provide a buffer against detrimental psychological and physical effects associated with severe or chronic stress. However, chronic stress can significantly impact an individual's ability to form and maintain relationships, negating the positive effects of these connections. Neurobiological systems responsible for relationship formation and maintenance, such as dopamine signaling within the nucleus accumbens (NAc), are susceptible to alteration following a stressful experience. Notably, corticotropin-releasing hormone (CRH) has been demonstrated to differentially modulate dopamine release within the NAc based on prior stress experience. That is, intra-NAc CRH release is appetitive and promotes intra-NAc dopamine release in a non-stressed state, but it becomes an aversive stimulus and uncouples from dopamine during chronic stress. The NAc receives CRHergic projections from the bed nucleus of the stria terminalis (BNST), which has been demonstrated to impact motivated behavior such as social interaction and fear aversion. In addition, it is well-documented that dopamine in the NAc promotes pair bonding in prairie voles (*Microtus ochrogaster*), and recently, we identified a role for BNST CRH in the regulation of pair bond formation. Therefore, BNST CRHergic activity may contribute to changes in both intra-NAc dopamine release and relationship formation, but a direct link has not been established. In this study, we utilized the social defeat model in the socially monogamous prairie vole to investigate the effect of social defeat on pair bond formation in both males and females and determine the role of BNST CRHergic activity in any changes observed. We observed that defeat leads to a sexually dimorphic, opposing effect on pair bond formation, in that defeat impaired bonding in males and accelerated bonding in females. We hypothesize that BNST CRHergic activity has both sex-dependent and stress-dependent effects on intra-NAc dopamine release and underlies the sex difference in pair bond abnormalities following defeat. The effect of the combination of defeat experience, bonding status, and BNST CRHergic activity using chemogenetic manipulation on NAc dopamine content is investigated with *in vivo* microdialysis and dopamine receptor activity through characterization of DARPP-32 levels and phosphorylation state. The results of this study may provide insight into the neurobiology contributing to the negative impact of stress on relationship formation.

Disclosures: M. Tickerhoof: None. **A.S. Smith:** None.

Poster

P588. Neurobiological Bases of Social Behavior

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Program #/Poster #: P588.07

Topic: F.02. Behavioral Neuroendocrinology

Support: Fulbright Colombia - Fulbright Scholarship Pasaporte a la ciencia
General Research Fund from The University of Kansas (KU) Office of Research

Title: Social defeat regulation of oxytocin-related signaling proteins in female prairie voles (Microtus ochrogaster)

Authors: *L. NERIO MORALES[1], A. S. SMITH[2];
[1]Pharmacol. and toxicology, The Univ. of Kansas, Lawrence, KS; [2]Pharmacol. and toxicology, Lawrence, Lawrence, KS

Abstract: Social anxiety disorder (SAD) is one of the most common and disabling anxiety disorders. SAD is highly prevalent in female population and characterized by generalized social avoidance often comorbid with other psychiatric diseases. Despite its high prevalence and adverse prognosis, the molecular mechanism behind this condition is poorly understood. The social defeat (SD) paradigm is the most representative animal model to study SAD and the underlying neural mechanisms. Particularly, SD in prairie voles (*Microtus ochrogaster*) advantageously allows the study of female subjects due to their spontaneous display of aggression towards unfamiliar conspecifics after pair bond. Recently, we have documented that SD robustly decreases oxytocin receptor (OTR) levels in limbic brain regions relevant for affiliative behaviors and stress in female prairie voles, suggesting a role of this neuropeptide in the etiology of SAD. Oxytocin stimulation of its receptor leads to increased intracellular calcium levels and activation of the mitogen-activated protein kinase (MAPK) pathway, this last one being previously associated with the regulation of the anxiolytic effects of oxytocin. Here, we used female prairie voles to study the effect of SD on oxytocin-related signaling proteins and their response to a social encounter. Preliminary data obtained from regions where OTR was previously reported as downregulated, suggests that defeat increases the phosphorylation state of CREB, a transcription factor involved in the alleviation of anxiety-like behaviors. The continuation of this study will include measuring upstream signaling pathways associated with CREB that are responsive to changes in OTR expression, namely, the MAPK pathway and calcium-dependent signaling pathways.

Disclosures: L. Nerio Morales: None. **A.S. Smith:** None.

Poster

P588. Neurobiological Bases of Social Behavior

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Program #/Poster #: P588.08

Topic: F.02. Behavioral Neuroendocrinology

Support: NE 911_3-1

Title: Real-time molecular imaging oDrosophila melanogaster males by quaf social driven aggression in ntitative single cell mass spectrometry

Authors: M. DIESNER[1], B. DREBES[2], H. ABERLE[3], ***S. NEUPERT**[2];
[1]Univ. of Cologne, Cologne, Germany; [2]Univ. of Kassel, Kassel, Germany; [3]Heinrich-Heine-Universität, Duesseldorf, Germany

Abstract: Aggression in the whole animal kingdom is strongly associated with acquisition and surveillance of resources such as food, territory, fight and mates. In both vertebrate and invertebrate, a number of genes have been identified as important players in aggression-associated behaviors. Most of them encode biogenic amines, neuropeptides, and their respective receptors with their second messenger cascades. In invertebrates, the majority of aggression-associated genes and signaling molecules were studied in the insect model *Drosophila melanogaster* via mutagenesis and direct labeling to allow for cellular analysis. The biogenic amine octopamine (OA), equivalent to norepinephrine in vertebrates, plays a key role in controlling aggression behavior. Two subpopulations of VUM/VPM neurons located in the gnathal ganglion have been shown to be required for regulation of aggression: (1) Three VUM/VPM neurons express the male specific transcription factor *fruM,* and (2) a small subset of 2-5 VMlb OA-expressing neurons. Here, we present a novel analytical approach to study the dynamics in up- and downregulation of OA on the single cell level in different behavioral contexts. For reliable VMlb cell identification, we used the Tdc2-GAL4 driver line crossed to a UAS-mCD8::GFP reporter line. First, individual VMlb-OA somata from both sexes at different ages were prepared for quantitative SC mass spectrometry as described in Diesner and Neupert (2018). Statistical analysis of OA titers in VMlbs revealed no differences between sexes and age groups. Next, we analyzed the OA titer in VMlbs between males reared in mixed groups, socially isolated males and pairwise regrouped flies. Statistical comparison of pairwise regrouped flies showed comparable OT titers as grouped males, while OA titers of isolated males were significantly lower. This novel analytical approach provides great potential for a quantitative analysis of the kinetics and functions of neurotransmitter signaling in neuronal circuits controlling behavior. [Supported by DFG grant NE 911/3-1 to SN]

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Poster

P588. Neurobiological Bases of Social Behavior

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Support: ERC Grant 856487
Minerva Foundation

Title: Sex differences and effects of pheromone processing in construction of dominance hierarchy in lab and wild mice

Authors: *N. ZILKHA, S. G. CHUARTZMAN, I. SOFER, Y. PEN, T. KIMCHI;
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Abstract: Social interactions in a group are comprised of multiple complex behaviors, and are crucial for the survival of most mammalian species. In recent years, groups of conspecific laboratory mice living together under semi-natural conditions have been studied by us and others, to examine the formation of social dominance hierarchy. In laboratory male mice, most researchers report the formation of solid, stable, hierarchies, while laboratory females present weaker and less stable organizations, if any.Here, we examine the role of pheromones detected through the vomeronasal system (i.e. TrpC2-mediated), in the formation of dominance hierarchies and phenotypic individuality in a group of mice, under ethologically relevant conditions, in both sexes. We analyzed social behaviors in groups of both lab and wild-derived mice using an automated semi-natural system. Moreover, we utilized our unique strain of backcrossed wild-derived TrpC2 knockout mice, which lack a functional vomeronasal organ (VNO). These mice possess a wild genetic background, and also cannot detect VNO-mediated pheromones, similar to laboratory TrpC2 knockouts, which do not present dyadic aggression.In inbred laboratory mice, we show that groups of males establish a solid dominance hierarchy within just 2 days, while females do not present any clear hierarchy throughout the experimental period. Surprisingly, TrpC2 knockout laboratory male mice also form a hierarchical structure, however it is delayed and appears to be more linear then despotic. In wild mice, we show for the first time that groups of female mice establish a strong, solid, dominance hierarchy, similar to that of males. We also show that in wild mice, genetic ablation of VNO-mediated pheromone processing in TrpC2 knockout mice does not seem to impair the construction of dominance hierarchy. Our results demonstrate the importance of examining social behaviors in wild mice, especially when for analysis of females and for deciphering sexual dimorphism. Moreover, our results indicate on further aspects of sensory perception in VNO-deficient mice, suggesting they utilize other pathways and/or modalities to detect social cues from conspecifics.

Disclosures: N. Zilkha: None. **S.G. Chuartzman:** None. **I. Sofer:** None. **Y. Pen:** None. **T. Kimchi:** None.

Poster

P588. Neurobiological Bases of Social Behavior

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P588.10

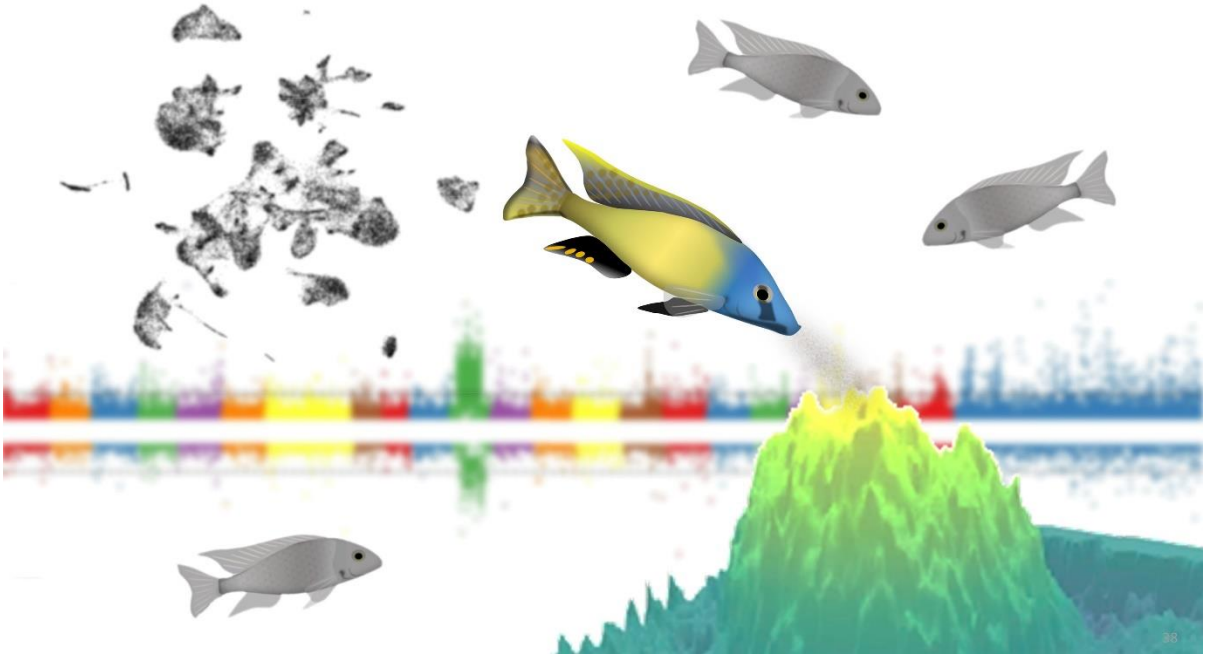
Topic: F.02. Behavioral Neuroendocrinology

Support: HFSP Grant RGP0052
NIH Grant R01GM101095
NIH F32GM128346

Title: Cellular substrates of a complex social behavior in cichlid fishes

Authors: *Z. V. JOHNSON, B. E. HEGARTY, G. W. GRUENHAGEN, T. J. LANCASTER, P. T. MCGRATH, J. T. STREELMAN;
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Abstract: A central goal of neuroscience is to understand the biological basis of complex behaviors. Although many complex behaviors have been linked to natural genetic variation in humans and other species, we know little about how these gene-behavior effects are coordinated by specific cell populations and molecular signaling systems. To address this gap, we integrate comparative genomics with brain region-specific single nuclei RNA-sequencing to link natural genetic variation to cell populations to complex social behavior in Lake Malawi cichlid fishes, a powerful vertebrate system for understanding natural behavioral variation and evolution. About 200 Lake Malawi species exhibit bower construction behavior, a goal-directed social behavior whereby males manipulate sand into large structures for courtship and mating over the course of many days. Species variation in bower construction behavior has been linked to natural genetic variation within a concentrated region on a single chromosome. Here, we 1) transcriptionally map the cellular landscape of the cichlid telencephalon, 2) link behavior-associated genomic variation to specific cell populations, and 3) functionally profile these populations during active bower construction behavior in *Mchenga conophoros* males. We identify robust signals of behavior-associated evolution, neuromodulatory signaling, neuronal activation, neurogenesis, and gene regulatory network architecture in neuronal and non-neuronal cell populations. Further, we show that the same cell populations exhibiting signatures of behavioral evolution are selectively activated during behavior. Lastly, we identify candidate genes, cell populations, and molecular signaling systems whose causal role in behavior can be investigated. Taken together, our work shows how single nuclei RNA-sequencing can be used to link natural genetic variation to behaviorally-relevant cell populations in any species system with a suitable genome sequence.



Disclosures: Z.V. Johnson: None. **B.E. Hegarty:** None. **G.W. Gruenhagen:** None. **T.J. Lancaster:** None. **P.T. McGrath:** None. **J.T. Streelman:** None.

Poster

P588. Neurobiological Bases of Social Behavior

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Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P588.11

Topic: F.02. Behavioral Neuroendocrinology

Support: Brain & Behavior Research Foundation, NARSAD Young Investigator Award 28298
Scott-Gentle Foundation

Title: Maternal lineage diet-induced gut dysbiosis and social dysfunction propagate across generations

Authors: C. M. DI GESÙ, L. M. MATZ, I. J. BOLDING, R. S. FULTZ, ***S. A. BUFFINGTON**;
Neuroscience, Cell Biology, & Anat., Univ. of Texas Med. Br., Galveston, TX

Abstract: Gut microbiota are emerging as powerful regulators of host physiology, including brain function and behavior. Diet-driven westernization of the gut microbiota, or microbiome, commonly seen in industrialized countries, is characterized by a significant reduction in

microbial diversity and accompanying loss of beneficial functionalities afforded to the host by a rich microbial ecosystem. Given that the maternal gut microbiome modulates fetal and early postnatal brain development, it is essential to understand the impact of westernization of the maternal gut microbiome on offspring brain development and behavior. We previously showed that maternal Western-style diet (mWd) in mice induces long-term, functional changes in the gut microbiome of male offspring that are causally related to autism-like social dysfunction and underlying deficits in the hypothalamic oxytocinergic-mesocorticolimbic dopaminergic social reward circuit. Here, using metagenomic 16S ribosomal RNA gene amplicon sequencing, we found that mWd similarly induces long-term dysbiosis of the female offspring (F_1) gut microbiome, despite their being transitioned to a regular diet upon weaning. In light of these data, we hypothesized that the enduring dysbiosis of the F_1 female gut microbiome may negatively impact neurodevelopment and behavior in the subsequent generation (F_2). Interestingly, while we observed a partial recovery of the diversity of the F_2 mWd lineage gut microbiome, we observed a complete loss of social preference in male, but not female, F_2 offspring, consistent with our findings in the F_1 generation and the strong male bias in autism. Furthermore, oxytocin levels in the paraventricular nucleus of the hypothalamus were reduced in the F_2 male offspring. Notably, post-weaning dietary probiotic supplementation of the F_2 males rescued their social dysfunction. Our data provide critical insight into the potential for Western diet-inflicted scars on the gut microbiome, particularly within the maternal lineage, and related host phenotypes to propagate across generations. More importantly, they suggest antenatal targeting of the maternal gut microbiome during pregnancy may be an innovative prophylactic approach to improving mental health outcomes in children.

Disclosures: C.M. Di Gesù: None. **L.M. Matz:** None. **I.J. Bolding:** None. **R.S. Fultz:** None. **S.A. Buffington:** None.

Poster

P588. Neurobiological Bases of Social Behavior

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P588.12

Topic: F.02. Behavioral Neuroendocrinology

Support: CONACYT CF-2020-6390

Title: Personality traits in a cohort of Mexican twins

Authors: *B. GARCÍA-VILCHIS[1], T. ROMÁN-LÓPEZ[2], E. ORTEGA-MORA[1], U. CABALLERO-SÁNCHEZ[1], E. CHIU-HAN[2], D. RAMÍREZ-GONZÁLEZ[2], O. ALDANA-ASSAD[3], A. TAPIA-ATILANO[1], S. PRADEL-JIMÉNEZ[1], D. ZENTENO-MORALES[1], Z. ESPINOSA-VALDES[1], X. LÓPEZ-CAMAÑO[2], M. RENTERÍA[3], A. MEDINA-RIVERA[4], S. ALCAUTER[2], A. RUIZ-CONTRERAS[1];

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Abstract: Corresponding authors: aleruiz@unam.mx, alcauter@inb.unam.mx. Twin studies aim at comparing the similarity, with respect to a selected trait, between monozygotic twins (MZ), and dizygotic twins (DZ). The comparison of traits between twins allows us to analyze the relative contribution of genetic and environmental factors in various phenotypes (Craig JM et al., 2020). Mexico created the first national twin registry in 2017, TwinsMX (Leon-Apodaca et al., 2019), with which we aimed to estimate the heritability of different traits among the Mexican population, which presents a genetic admixture, different from that observed in other regions of the world (Moreno-Estrada et al., 2014). Here we show results of the Big Five Inventory (Extraversion, Agreeableness, Conscientiousness, Neuroticism and Openness) applied to twins currently registered in TwinsMX. **Methods**. Twin pairs were recruited through different media to remotely respond to the Big Five Inventory. **Results**. Currently, we have the response of 96 twins (MZ n = 74; DZ n = 22) who completed the inventory. In the sample, a higher proportion of female participants (78%) and young people between 21-30 years old (44%) is observed. It is a small sample from which preliminary conclusions about the population can be drawn. Here we present the correlation between MZ and DZ in the following traits: Extraversion:); Agreeableness:); Conscientiousness:); Neuroticism:); Openness:). **Conclusions**. These preliminary results represent a first advance in the study of personality trait heritability using TwisnMX data, showing that extraversion, neuroticism and openness seems to reflect a higher genetic component compared to the agreeableness and conscientiousness. However, it is necessary to increase the sample size to calculate a reliable index of heritability for each personality trait for Mexican population. Keywords: twins, big five, trait, personality, heritability. GRANT: CONACYT CF-2020-6390.

Disclosures: B. García-Vilchis: None. **T. Román-López:** None. **E. Ortega-Mora:** None. **U. Caballero-Sánchez:** None. **E. Chiu-Han:** None. **D. Ramírez-González:** None. **O. Aldana-Assad:** None. **A. Tapia-Atilano:** None. **S. Pradel-Jiménez:** None. **D. Zenteno-Morales:** None. **Z. Espinosa-Valdes:** None. **X. López-Camaño:** None. **M. Rentería:** None. **A. Medina-Rivera:** None. **S. Alcauter:** None. **A. Ruiz-Contreras:** None.

Poster

P608. Stress: Translationally Relevant Rodent Models

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P608.01

Topic: F.04. Stress and the Brain

Support: NIH RF1 AG057884-01 and Diversity Supplement
NIH T32 2T32GM008152-31 for Northwestern MSTP

Title: Chronic isolation and acute restraint stress do not affect memory and affective behavior in 5XFAD mice

Authors: *S. DOMINGUEZ[1], A. LOCCI[3], H. DONG[2];
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[3]Psychiatry and Behavioral Sci., Northwestern Univ., Chicago, IL

Abstract: Introduction: Previous studies from our group and others suggest that physical and social stress are both risk factors of Alzheimer's Disease (AD). In this study, we aimed to determine whether cognitive decline, affective behavior, and biochemical markers of AD were significantly impacted in a sex-dependent manner by chronic isolation or acute physical restraint stress in an AD mouse model. **Methods:** Firstly: 5XFAD mice were either singly or group-housed (3-5/cage) beginning at wean age (21-23d) for 12 months. After isolation stress, all mice (n=12-14/sex/condition) underwent behavioral analyses for memory [Novel Object Recognition (NOR), Y-Maze, Morris Water Maze (MWM)], anxiety-like behavior [Open Field Test (OFT), Light Dark Test (LD)], and depressive-like behavior [Tail Suspension Test (TST)]. Secondly: 12m 5XFAD and wild-type (WT) littermates underwent one instance of 24h of restraint stress or no stress (n=10/sex/condition) prior to behavioral analyses for memory (Y-maze, MWM), anxiety-like behavior (OFT, LD), and depressive-like behavior (TST). Then, brain tissues were collected for biological analyses. **Results:** 12-month-old 5XFAD mice displayed memory deficits and depressive-like behavior, but neither chronic isolation nor 24h restraint stress resulted in significant differences compared to non-stressed groups. Specifically, Y-maze showed a significant effect of genotype ($p<0.0001$) regardless of stress condition, but isolation ($p=0.5206$) and 24h restraint ($p=0.6404$) had no effect on memory in either genotype. Latency to reach the hidden platform in MWM was significantly longer in 5XFAD mice ($p<0.0001$) with an effect of sex ($p=0.0084$) but not stress (isolation $p=0.4489$, 24h restraint $p=0.9360$). In OFT, WT male mice exposed to 24h restraint stress had a significant reduction in the time spent in the center ($p=0.0229$); however, isolated 5XFAD mice and non-isolated 5XFAD mice did not significantly differ (isolation $p=0.8853$). While 5XFAD mice spent significantly more time immobile during the TST ($p<0.0001$), neither isolation ($p=0.0875$) nor 24h restraint ($p=0.1865$) were significant. In the LD, isolated ($p=0.8985$) and 24h restraint ($p=0.7031$) mice had no significant differences compared to non-stressed mice. Biological analyses are underway. **Conclusions:** Our preliminary data has confirmed that 5XFAD mice display some memory deficits and depressive-like behavior at 12m, however, the chronic and acute stress paradigms we selected did not affect such behaviors. These results are unexpected and future studies are needed to confirm whether 5XFAD mice with the C57 genetic background are more resilient to physical and social stress.

Disclosures: S. Dominguez: None. **A. Locci:** None. **H. Dong:** None.

Poster

P608. Stress: Translationally Relevant Rodent Models

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P608.02

Topic: F.04. Stress and the Brain

Support: T32MH087004
R01NS107512

Title: Social stress alters behavioral responses, synaptic properties and intrinsic excitability in nucleus accumbens of knock-in mice carrying the Parkinson's disease-linked LRRK2-G2019S mutation

Authors: *C. A. GUEVARA, B. A. MATIKAINEN-ANKNEY, S. J. RUSSO, D. L. BENSON, G. W. HUNTLEY;
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Abstract: Parkinson's disease (PD) is associated with cognitive and psychiatric (anxiety, depression) non-motor symptoms that appear early, are initially independent of dopamine neuron loss and are poorly understood. The LRRK2-G2019S mutation is the most common genetic cause of late-onset PD. The risk for both PD and depression is increased by behavioral stress. To probe relationships between PD mutation, stress, and neural circuit modifications, we subjected young adult (2-3 mo) male wildtype (WT) and G2019S knockin mice to acute (1d) social defeat stress (SDS) followed by whole-cell recordings from striatal projection neurons in the nucleus accumbens (NAc)-- an area enriched in LRRK2 and known to regulate stress and depression-like responses--in order to interrogate the impact of 1d-SDS on synaptic function and intrinsic membrane properties. Following 1d-SDS, G2019S mice were significantly more socially avoidant compared to 1d-SDS WT mice. Subsequent whole-cell recordings in NAc revealed that mutant SPNs failed to exhibit changes in intrinsic excitability seen in 1d-SDS WT SPNs, but instead showed synaptic changes that WT SPNs lacked. Together, these data indicate that G2019S drives a temporally-evolving set of cellular and synaptic adaptations to behavioral stress that differ from WT and may impact onset of psychiatric symptoms in human PD patients. Identifying synaptic and cellular adaptations to stress may reveal novel targets for ameliorating mood-related and cognitive symptoms associated with PD.

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Poster

P608. Stress: Translationally Relevant Rodent Models

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Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P608.03

Topic: F.04. Stress and the Brain

Support: University of Richmond Arts & Sciences Summer Research Program

Title: Neurobiological differences between laboratory and wild-trapped Rattus Norvegicus: Implications for translational value of preclinical psychiatric models and therapeutic interventions

Authors: J. JACOB[1], S. WATANABE[1], N. GONZALES[1], J. RICHARDSON[1], E. G. PLOPPERT[1], G. LAHVIS[2], A. B. SHIELS[3], S. WENGER[1], K. SAVERINO[1], J. BHALERAO[1], B. CROCKETT[1], E. BURNS[1], O. HARDING[1], K. STENGER[1], ***K. LAMBERT**[1]; [1]Univ. of Richmond, Richmond, VA; [2]Harvard Law Sch., Boston, MA; [3]Natl. Wildlife Res. Ctr., Fort Collins, CO

Abstract: For over a century, preclinical research has utilized numerous laboratory-bred strains of *Rattus norvegicus*. Although research with these models has produced valuable information, in biomedical disciplines such as psychiatric research, rodent models continue to lack predictive validity. Despite the annual use of tens of millions of rodents in U.S. laboratories, few studies have compared laboratory rodent strains with their wild counterparts. Recognizing the importance of environmental influences on neural functions, laboratory habitats are severely restricted in comparison to the dynamic, sensory-rich habitats of wild rats. In the current study, we compared two wild rat populations (i.e., each from an urban and rural environment) to a common outbred laboratory strain, the Long Evans (LE) rat (Envigo, Inc). Laboratory rats were weight-and sex-matched to the wild urban rats. Wild urban rats were live-trapped in Richmond, VA, and were euthanized so their brains could be immediately extracted and post-fixed. Additional morphological measures were collected including spleen, adrenal, and body weights; further, fecal boli were collected for corticosterone and DHEA analyses. Brains were dissected and prepared for histological analysis, including isotropic fractionation, to determine cell counts in the cell-dense cerebellum, with ongoing assessments of cytoarchitectural profiles in the hippocampus and amygdala as well as cytokine levels in spleen tissue. Whole brains of the rural/farm rats were post-fixed and sent to our lab for histological analyses. LE rats were acclimated to the vivarium for one week in standard laboratory conditions before tissue collection as described for the wild rats. Fecal samples were collected from the lab rats immediately upon arrival to capture transport stress to compare to trap-related stress of wild rats. Wild rat adrenal glands were 3x larger than the lab rats ($p=.001$) and, focusing on the spleen weights, a significant interaction ($p=.045$) indicated that the 4x increase in female wild spleen weights compared to female lab rat spleens was larger than the 2x increase observed in wild male spleen weights compared to lab rats. LE cerebella ($N = 10$) contained significantly fewer cells than both urban ($N = 11$, $p = 0.00002$) and farm rats ($N = 14$, $p = 0.002$) rats (i.e., 20% and 15% fewer, respectively). Of specific relevance for stress-related preclinical models, stressed corticosterone levels were 5x higher in urban rats compared to laboratory rats ($p=.025$).

Additional research is necessary to explore the clinical relevance of laboratory and wild rat comparisons to determine the translational value of rodent neuropsychiatric models.

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Poster

P608. Stress: Translationally Relevant Rodent Models

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P608.04

Topic: F.04. Stress and the Brain

Support: NARSAD Young Investigator Grant from the Brain & Behavior Research Foundation 29503
NIMH Grant 1R21MH122965

Title: Behavioral consequences of a traumatic experience in mice

Authors: *I. D. B. WEBER[1], R. HEN[2], G. F. TURI[1];
[1]Systems Neurosci., New York State Psychiatric Inst., New York, NY; [2]Neurosci. and Psychiatry, Columbia Univ., New York, NY

Abstract: Though fear is an adaptive behavior essential for survival, overexpressed fear can cause anxiety in situations where it is not called for and reduce quality of life. Patients with post-traumatic stress disorder (PTSD) have difficulties distinguishing similar contexts containing cues related to a traumatic experience and are less able to determine which are safe or threatening. The hippocampus and specifically the dentate gyrus subarea have long been implicated in the pathogenesis of PTSD, possibly due to its role in pattern separation, the network-level process which underlies the ability to distinguish highly similar contexts. Thus, we hypothesized that mice which experience trauma and develop PTSD-like behavior will also have impairments in behaviors that depend on intact hippocampal function. To test this idea, our first aim was to develop a high-throughput behavioral profiling pipeline which can be used to characterize PTSD-like and hippocampal dependent mouse behavior before and after the traumatic experience with the least amount of human interference and bias. To subject mice to trauma, we used the stress-enhanced fear learning (SEFL) footshock protocol. Our behavioral profiling battery included the open field task and elevated plus maze to assess anxiety-like behavior, as well as object recognition and spontaneous alternation tests for assessing memory and the functional integrity of the hippocampus. To subject mice to a traumatic experience, we used the stress-enhanced fear learning (SEFL) footshock protocol. To analyze how trauma affects

behavior in these tasks, we implemented the DeepLabCut package for tracking mouse body parts in recorded video and wrote additional code to analyze the tracking results. In spite of the frequent occurrence of negative experiences in humans, the overall lifetime prevalence of PTSD in the US population is about 10%, which suggests that factors other than trauma are also involved in the pathogenesis of PTSD. Thus, we identified a subset of SEFL treated mice exhibiting pronounced freezing in a fear conditioned context even after repeated exposure to the context without shock. These mice freeze 40% of the time after 4 exposures, as opposed to the resilient population which freezes only 10% of the time. We are planning experiments to investigate network-level disruptions of contextual discrimination in the dentate gyrus of mice expressing this behavioral phenotype. A characterization of the network properties underlying pattern separation and contextual discrimination which are disturbed in a PTSD-like state will improve our understanding and ability to find endophenotypes of PTSD based on circuit components.

Disclosures: I.D.B. Weber: None. **R. Hen:** None. **G.F. Turi:** None.

Poster

P608. Stress: Translationally Relevant Rodent Models

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Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P608.05

Topic: F.04. Stress and the Brain

Support: Hope for Depression Research Foundation

Title: Role of the ventral hippocampal circuit in the responses to chronic stress, acetylcarnitine and ketamine

Authors: *B. BIGIO[1], J. DOBBIN[1], O. BARNHILL[1], D. MILLER[1], R. HEN[4], E. J. NESTLER[5], B. S. MCEWEN[2], C. NASCA[3];
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Abstract: Chronic stress is a main risk factor for major psychiatric disorders, including depression. In rodent models, administration of acetylcarnitine (LAC) leads to a rapid and persistent antidepressant-like response by increasing a chronic stress-induced suppression of acetylation of specific histone markers and the related expression of key genes for synaptic plasticity in ventral dentate gyrus (vDG). In patients with depression, LAC levels are decreased. Yet, the circuit level mechanisms underlying the rapid antidepressant-like responses of LAC remains to be determined. Here, we used a chemogenetic, behavioral and pharmacological approach to test the role of the ventral hippocampal circuit in the responses to LAC as compared

to ketamine at chronic stress models. Our preliminary data showed that either administration of ketamine (10 mg/kg, i.p. injection) or LAC (100 mg/kg, i.p. injection) rescues depressive-like phenotypes at both the chronic restraint stress (CRS) and social defeat stress (SDS). Next, we used Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) approaches to suppress neuronal firing of the ventral hippocampal circuit in transgenic mice expressing Cre recombinase under the control of the mouse calcium/calmodulin-dependent protein kinase II alpha (Camk2a) promoter (Camk2a-Cre+ mice). Preliminary data showed that chemogenetic silencing of neuronal firing of the ventral hippocampal circuit blunted the action of LAC on the amelioration of CRS-induced behavioral deficits. In all, these findings support the critical role of the ventral hippocampus in stress responses and in the responses to the novel epigenetic modulator of glutamatergic function.

Disclosures: B. Bigio: None. **J. Dobbin:** None. **O. Barnhill:** None. **D. Miller:** None. **R. Hen:** None. **E.J. Nestler:** None. **B.S. McEwen:** None. **C. Nasca:** None.

Poster

P608. Stress: Translationally Relevant Rodent Models

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Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P608.06

Topic: F.04. Stress and the Brain

Support: NIMH P50MH096890
NIMH R01MH051399
Hope for Depression Research Foundation

Title: The Expression of Key Hub Genes within the Nucleus Accumbens Mediates Resilience to Chronic Stress.

Authors: *T. GYLES[1], E. M. PARISE[2], A. GODINO[3], C. J. BROWNE[4], Z. S. LORSCH[5], E. J. NESTLER[6];
[1]Icahn Sch. of Med. At Mount Sinai Grad. Training Program In Neurosci., New York, NY; [2]Dept. of Neurosci., Ichan Sch. of Med. At Mount Sinai, New York, NY; [3]Nash Family Dept. of Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY; [4]Neurosci., Mount Sinai Icahn Sch. of Med., New York, NY; [5]Neurosci. and Friedman Brain Inst., Icahn Sch. of Med. At Mount Sinai, New York, NY; [6]Icahn Sch. Med. At Mount Sinai, New York, NY

Abstract: The Expression of Key Hub Genes within the Nucleus Accumbens Mediates Resilience to Chronic Stress.
T. GYLES[1], E. M. PARISE[1], A. GODINO[1], C. J. BROWNE[1], Z. S. LORSCH[1], E. J. NESTLER[1];
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Medicine at Mount Sinai New York, NY
Major depressive disorder (MDD) is the leading cause of disability and a leading contributor to suicide according to the World Health Organization. Unfortunately, close to half of all patients diagnosed with MDD are at least partly resistant to antidepressant treatment. Notably, both genetic and environmental factors, including exposure to chronic social stress, play a role in the development of MDD and related syndromes. The chronic social defeat stress (CSDS) paradigm in mice has proven to be a highly useful animal model for studying depression-related behavioral abnormalities. Importantly, this paradigm allows for the identification of animals that succumb to the effects of the stress, termed susceptible, from those that do not, termed resilient. To better understand the potential genes underlying the resilient phenotype, we performed RNA-sequencing on mice exposed to CSDS and compared gene expression changes within the nucleus accumbens (NAc) between resilient and susceptible mice. Using weighted gene co-expression network analysis (WGCNA), we identified gene expression patterns that are associated uniquely with resilience. These data were then evaluated using an undirected key driver analysis that allows for the reconstruction of accurate cellular networks (ARACNE) based on gene-gene correlations. We identified three genes (*Gprin1*, *Bcr*, and *Stx1a*) that were predicted to be key drivers (predicted to regulate other genes) within a highly significant, resilient-specific module. We are now investigating how changes in the expression of these genes could lead to changes in depressive-like behavior. To test this, multiple cohorts of mice were exposed to CSDS or another stress paradigm, chronic variable stress, and mRNA expression levels of these target genes were assessed. We also tested the functional consequences of virally manipulating these genes following prior to CSDS. Evidence from these studies suggests that overexpression of each of these genes in NAc neurons produces a pro-resilient effect. Current work is investigating these molecular mechanisms in a cell-type-specific manner within the NAc. Together, these studies are providing novel insight into the molecular basis of stress resilience.
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Disclosures: T. Gyles: None. **E.M. Parise:** None. **A. Godino:** None. **C.J. Browne:** None. **Z.S. Lorsch:** None. **E.J. Nestler:** None.

Poster

P608. Stress: Translationally Relevant Rodent Models

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Program #/Poster #: P608.07

Topic: F.04. Stress and the Brain

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The European Union Project(KARTRAP), Grant/Award Number: QLG3-CT-2001-02089
Medical Student Research Programme at the Faculty of Medicine, University of Oslo

Title: Changes in concentrations of NMDA receptor subunit GluN2B, Arc and syntaxin-1 in dorsal hippocampus Schaffer collateral synapses in a rat learned helplessness model of depression

Authors: *S. DAVANGER[1], S. HUSSAIN[1], M. BIELER[1,2], E. S. B. DAALAND[1], M. M. MIRRIONE[3,4], F. A. HENN[4,5];
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Abstract: Major depressive disorder involves changes in synaptic structure and function, but the molecular underpinnings of these changes are still not established. In an initial pilot experiment, whole-brain synaptosome screening with quantitative western blotting was performed to identify synaptic proteins that may show concentration changes in a congenital rat learned helplessness model of depression. We found that the N-methyl-D-aspartate receptor (NMDAR) subunits GluN2A/GluN2B, activity-regulated cytoskeleton-associated protein (Arc) and syntaxin-1 showed significant concentration differences between congenitally learned helpless (LH) and nonlearned helpless (NLH) rats. Having identified these three proteins, we then performed more elaborate quantitative immunogold electron microscopic analyses of the proteins in a specific synapse type in the dorsal hippocampus: the Schaffer collateral synapse in the CA1 region. We expanded the setup to include also unstressed wild-type (WT) rats. The concentrations of the proteins in the LH and NLH groups were compared to WT animals. In this specific synapse, we found that the concentration of NMDARs was increased in postsynaptic spines in both LH and NLH rats. The concentration of Arc was significantly increased in postsynaptic densities in LH animals as well as in presynaptic cytoplasm of NLH rats. The concentration of syntaxin-1 was significantly increased in both presynaptic terminals and postsynaptic spines in LH animals, while pre- and postsynaptic syntaxin-1 concentrations were significantly decreased in NLH animals. These protein changes suggest pathways by which synaptic plasticity may be increased in dorsal hippocampal Schaffer collateral synapses during depression, corresponding to decreased synaptic stability.

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Poster

P608. Stress: Translationally Relevant Rodent Models

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P608.08

Topic: F.04. Stress and the Brain

Support: This work was supported by National Fund for Scientific, Technological, and Technological Innovation Development) (FONDECYT) and Word Bank (BM) grant # 025-2019 Fondecyt-BM

Title: A novel fatty acid amide hydrolase inhibitor as a potential antidepressant and its effect on gene expression in BALB/c mice acute stress model

Authors: *C. MEDINA-SALDIVAR, S. CRUZ-VISALAYA, G. E. PARDO, J. M. IGLESIAS-PEDRAZ, L. F. PACHECO-OTALORA;
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Abstract: Background Endocannabinoid modulators has recently gained relevance as a potential treatment of depression due to its particular mechanism of action that differs from the classic antidepressants. Inhibitors of the fatty acid amide hydrolase (FAAH) have demonstrated antidepressant effects in the murine model by increasing the bioavailability of anandamide in the central nervous system. In the present study, we have used a novel compound (MCH1) derivate from the Peruvian plant *Lepidium meyenii* (maca) that shown an *in vitro* FAAH inhibitory activity. Our goal was to evaluate the antidepressant effect of different MCH1 doses i.p administered in mice that were then exposed to acute stress using shawn mice as controls. Also, we evaluate the effect of the optimal dose of MCH1 on the gene expression levels of *FAAH*, *Cnr1* and *NAPE-PLD* in the dentate gyrus of treatment and controls mice. **Methods** 25 male BALB/c mice (2-month-old) were randomly assigned in groups of 5. Each group received a single dose of vehicle (1: 1: 18 DMSO / polyethylene glycol 600 / 0.9% saline (shawn)), 3 mg/kg MCH1, 10mg/kg MCH1, 30mg/kg MCH1 or 20mg/kg fluoxetine. After one hour, mice were exposed to a forced swim test (acute stressor) and the immobility and latency time evaluated. One hour after the test, the brains were collected, freeze at -80°C and cryostate-cutted at 500 µm. The hippocampus was 16G micropunched at dentate gyrus of both sides, and RNA was extracted by TRIzol method. Genomic DNA was removed by DNase I before cDNA generation. Freshly prepared cDNA was used to perform qPCR. **Results** Tukey's post-hoc analysis showed that the administration of 10mg/kg (59 sec, $p < 0.01$) and 30mg/kg (77.40sec, $p < 0.05$ MCH1 vs 151.8 sec vehicle) of MCH1 reduced the immobility time of mice significantly, reaching the maximum effect with 10mg/kg. Though the administration of MCH1 showed a trend of increasing immobility latency in the three MCH1 groups, this was not significant ($p = 0.2107$). Thus, 10mg/kg MCH1 group was selected for gene expression analysis. Our preliminary qPCR data reveals that, after MCH1 treatment there is a significant increase in *FAAH* expression while observing a reduction in *Cnr1* and *NAPE-PLD* expression levels compared to vehicles. **Conclusion** The administrations of MCH1 have antidepressant activity in an acute model. The MCH1 dose of 10 mg/kg showed the most significant antidepressant effects. This antidepressant effect could be linked with the inhibitory effect of MCH1 on FAAH activity. The expression levels of *Cnr1* and *NAPE-PLD* was significantly reduced, suggesting a negative feedback

possible as an over signaling of anandamide as consequence of FAAH inhibition, irrespective of mRNA upregulation.

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Poster

P608. Stress: Translationally Relevant Rodent Models

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Program #/Poster #: P608.09

Topic: F.04. Stress and the Brain

Title: Predator odor (TMT) exposure modifies alcohol interoceptive sensitivity and differential gene expression in the insular cortex

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Abstract: Post-traumatic stress disorder (PTSD) confers enhanced vulnerability to develop problem drinking or AUD co-morbidity. Alcohol produces interoceptive (subjective) stimulus effects that likely inform drug intake by driving drinking or signaling satiety. Therefore, understanding the role of stress on alcohol interoceptive sensitivity is a clinically relevant question. The present studies used exposure to the predator odor 2,5-dihydro-2,4,5-trimethylthiazoline (TMT) to model aspects of a stressor relevant to PTSD. A Pavlovian drug discrimination procedure was employed to train rats to respond to a conditioned stimulus (light) to procure a sucrose reward (unconditioned stimulus) conditional on the interoceptive effects of alcohol. That is, male, Long-Evans rats were trained to discriminate the interoceptive effects of alcohol (2.0 g/kg, i.g.) from water. Upon stable discrimination, rats received a single, inescapable TMT exposure and then remained undisturbed in their home cage for 2 weeks prior to interoceptive sensitivity testing. Prior TMT exposure did not affect interoceptive effects of alcohol at 2 g/kg but potentiated the stimulus effects of 1 g/kg of alcohol. Because the interoceptive effects of 1 g/kg alcohol are predominantly mediated by GABA receptor potentiation, a substitution experiment using pentobarbital was conducted and showed potentiated alcohol-like stimulus effects in the TMT group compared to controls, confirming a GABAergic adaptation. Next, rats underwent extinction training by removing the sucrose reward, uncoupling the unconditioned stimulus (sucrose) from the conditioned stimulus (light) while under the alcohol interoceptive state at the training dose (2 g/kg). Both the TMT and control groups showed identical within-session extinction, but the TMT group showed persistence in responding to the first light presentation between sessions compared to controls that showed normal between-session extinction. Gene expression analyses 2 weeks after a TMT

exposure in a separate group showed that the inhibitory cell marker *GAD1* gene expression was elevated in the insular cortex, suggesting some GABA receptor adaptation, which may underlie the potentiated sensitivity of 1 g/kg alcohol and pentobarbital in the TMT group. Together, these data begin to elucidate mechanisms that may underlie stressor-induced adaptations to alcohol interoceptive sensitivity. Supported by: AA026537

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Poster

P659. Threat/Fear: Neural Circuits IV

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Program #/Poster #: P659.01

Topic: G.01. Fear and Aversive Learning and Memory

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NARSAD Young Investigator
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National Institute of Mental Health K08MH122733

Title: Prefrontal cortex norepinephrine represents predictability of aversive events.

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[1]Yale Univ. Interdepartmental Neurosci. Program, New Haven, CT; [2]Psychiatry, [3]Dept. of Psychiatry, Yale Univ., New Haven, CT; [4]Peking Univ., Beijing, China

Abstract: Animals must learn to predict levels of threat in environments containing uncertainty to respond with appropriate defensive behaviors. Neuromodulators such as norepinephrine (NE) have been proposed to mediate predictions under uncertain conditions as well as stress-related disorders such as anxiety disorders and post-traumatic stress disorder, which arise from irresolvable uncertainty. Both acetylcholine and dopamine have been shown to represent predictive signals during both appetitive and aversive learning. However, investigation of the role of norepinephrine in threat prediction has been limited by the lack of high-resolution measurements of NE. Models in which NE release in PFC represents threat, threat prediction errors, or valence-independent uncertainty may help to resolve the role of NE in this structure. To that end, we expressed the fluorescent norepinephrine sensor GRAB-NE2h is the dorsomedial prefrontal cortex (dmPFC) or in multiple brain regions simultaneously and measured norepinephrine release via fiber photometry. We measured norepinephrine release during fear conditioning while varying the temporal delay between cues and aversive outcomes. NE release transients are synchronous with footshock, and NE release during threat-predictive cues increases across multiple cue presentations regardless of temporal delay. However, amplitude and temporal characteristics of NE release are modulated by temporal delay. Cue-evoked NE

persists in the absence of cue-shock pairings but extinguishes over repeated cue presentations, which may be consistent with plasticity in the locus coeruleus. Cue-evoked NE release amplitude scales with time-discounted magnitude of predicted aversive events supporting a hypothesis in which NE release may correspond to threat level. Multi-region fiber photometry shows heterogeneous NE dynamics in several brain regions, but consistent shock and cue evoked elevations in release. Ongoing studies will examine the role of cue and shock evoked dmPFC NE in aversive learning, as well as the role of temporal and probabilistic unpredictability. Determining the role of norepinephrine in prediction of threats of varying certainty will illuminate computational processes underlying anxiety and anxiety disorders.

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Poster

P659. Threat/Fear: Neural Circuits IV

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Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH T32 MH065214
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Title: Behavioral and dopaminergic signatures during chronic social defeat stress that predict resilience

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Abstract: Chronic stress can have lasting adverse consequences in some individuals, yet others exhibit resilience. While molecular and intrinsic neural properties of the midbrain dopamine (DA) system differ in susceptible and resilient individuals following stress, if and how DA activity during the stressful experience itself differentiates susceptible and resilient individuals is an open question. To address this gap, we chronically record behavior and DA neural activity in the projections to the nucleus accumbens (NAc, signal reward) and tail of the striatum (TS, signals threat) during chronic social defeat stress. We find distinct behavioral and neural signatures of resilience and susceptibility. During stress, susceptible mice have lower NAc DA activity at the onset of aggressor encounters and higher NAc DA at the offsets during and after stress. In contrast, resilient mice exhibit higher NAc DA in proximity to the aggressor during and after defeat, exhibit more fighting back behavior during defeat, and have increased NAc DA

activity during this specific behavior. To test whether this specific timing of NAc DA activity is sufficient for producing resilience, we perform closed-loop (fight-back-triggered) and open-loop optogenetic stimulation of NAc DA neurons during stress. We find that both closed- and open-loop stimulation are pro-resilient, which suggests that increasing DA and positive valence generally in the aggressor interaction context is enough to protect against stress-susceptibility.

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Poster

P659. Threat/Fear: Neural Circuits IV

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Program #/Poster #: P659.03

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIMH Grant R21MH116242
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Title: Orexinergic projections to VTA and amygdala promote active avoidance

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Abstract: Proactive strategies for coping with threats are essential to survival. Identifying the neuromodulatory mechanisms that orchestrate survival behaviors is of critical importance to understanding fear- and stress-coping in health and disease. Perifornical (PFH) and lateral hypothalamus (LH) neurons expressing orexin (hypocretin) peptides project throughout the brain and mediate functions critical for survival behaviors including vigilance, attention, and action selection. Here we delineate the role of two targets of the hypothalamic orexin system using a model of proactive threat-coping behavior—signaled active avoidance (SigAA). Based on appetitive instrumental studies and physiology findings, we hypothesized that orexin projections to the central nucleus of the amygdala (CeA) would oppose restrictive freezing reactions, thus permitting proactive SigAA responses, and projections to the ventral tegmental area (VTA) would invigorate SigAA. Sprague Dawley rats received infusions of an orexin-specific viral vector containing an inhibitory opsin (AAV1-Ple112-Arch3.0-eYFP) into the PFH/LH, and optic fibers were implanted in the CeA or VTA. Following a 6-8 week incubation, rats were trained in the SigAA task. Animals received one Pavlovian trial (60 s white noise warning signal (WS) paired with an inescapable foot-shock (1.0/0.7 mA males/females; 0.5 s). For all remaining trials, if animals shuttled during the WS (an avoidance response), a feedback (FB) tone was delivered

(5 s, 80 dB), which served as a safety signal. Rats received 15 trials per day until reaching 80% avoidance responses per session. During test sessions, orexin-->CeA or orexin--> VTA axon terminals were inhibited either during the WS or the FB. Photoinhibition (green laser 532 nm, 10 mW) occurred during shock-free extinction tests. Impairments were observed in avoidance responses and avoidance latency when inhibiting orexin-->CeA or orexin-->VTA axon terminals. These results suggest that the orexin system suppresses passive reactions (freezing) and promotes adaptive instrumental actions (active avoidance) via two separate neural pathways, highlighting a novel role for the orexin system as a 'master modulator' of proactive coping behavior.

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Poster

P659. Threat/Fear: Neural Circuits IV

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Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P659.04

Topic: G.01. Fear and Aversive Learning and Memory

Support: ZIAMH002928

Title: The amygdala's role in learning from gains and losses

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Abstract: Adaptable behavior requires animals to learn the values of different stimuli to gain rewards and avoid losses. Most prior work has studied learning from rewarding outcomes. However, different learning environments, such as environments with aversive outcomes, may affect the animal's ability to learn. Therefore, we developed a task that allowed us to examine the neural systems involved with learning from both gains and losses. Previous research using this task has found that animals with ventral striatum (VS) lesions had deficits only when choosing between two rewarded outcomes, suggesting that different neural circuitry may underlie learning from losses. The amygdala is a prime candidate for this role because it plays a role in aversive learning, conditioned reinforcement, and visual stimulus-based reinforcement learning. Additionally, the amygdala projects to additional areas that are involved with coding value representations, driving motivated behavior, and processing aversive emotions. To investigate the role of the amygdala in learning from gains and losses, we compared rhesus monkeys (*Macaca mulatta*) with excitotoxic amygdala lesions (n = 4) with unoperated controls (n = 4) on a task in which monkeys chose between two pictures with associated gains and losses of tokens, which were presented visually on the screen. These tokens were conditioned reinforcers that

were periodically cashed out for a juice reward. They learned to choose cues associated with gains and avoid cues associated with losses of tokens. We ran four experiments. In the first, we introduced four images with deterministic outcomes of +2, +1, -1, and -2 tokens. The second task had an additional cue with a value of 0, resulting in five deterministic outcomes of +2, +1, 0, -1, and -2 tokens. The third task was a stochastic task with the four original cues. There was a 75% chance of receiving the original tokens and a 25% of not receiving any tokens. The fourth task resembled the first experiment but with -2 tokens substituted with -4 tokens, which acted as a large token loss. We found that token gains were appetitive and token losses were aversive, and that monkeys performed well on conditions with gains paired with losses. Additionally, monkeys learned to select the 0 token cue when it was paired with a loss cue. On the 0 token task, monkeys with amygdala lesions performed better than controls. Further, a reinforcement learning model with one parameter for each cue best fit the data. With this model, we found that lesion animals performed better with the +1 cue in the 0 token experiment, and this drove the significant difference between groups.

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Poster

P659. Threat/Fear: Neural Circuits IV

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Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P659.05

Topic: G.01. Fear and Aversive Learning and Memory

Support: OGS
NSERC
CIHR

Title: Hippocampal-hypothalamic circuit controls context-dependent innate defensive responses

Authors: *J. BANG[1], J. SUNSTRUM[4], D. GARAND[2], M. A. WOODIN[1], W. INOUE[4], J. KIM[3];
[2]Cell and Systems Biol., [3]Psychology, [1]Univ. of Toronto, Toronto, ON, Canada; [4]Univ. of Western Ontario, London, ON, Canada

Abstract: Preys use their memory - where they sensed a predatory threat and whether a safe shelter is nearby - to dynamically control their survival instinct to avoid harm and reach safety. However, it remains unknown which brain regions are involved, and how such top-down control of innate behavior is implemented at the circuit level. Here, we show that the anterior hypothalamic nucleus (AHN) is best positioned to perform this task as an exclusive target of the hippocampus (HPC) within the medial hypothalamic defense system. Direct AHN activation is

sufficient to evoke robust defensive responses (running, jumping, freezing) in the absence of threat and can act as an unconditioned stimulus in the conditioned place aversion test. In contrast, the HPC-AHN activation induced escape-associated locomotion in the absence of threat yet induced goal-directed escape when a shelter is available. Next, we inhibited HPC-AHN to test whether the pathway is necessary for context-dependent escape responses. Inhibition of HPC-AHN impaired avoidance of predator associated context and reduced escape runs to the safe context. When escape response is dependent on the availability of a shelter, we found that the HPC-AHN inhibition impaired efficiency (i.e. speed, escape linearity) and accuracy of shelter-oriented escapes upon ultrasound cue. Selective optogenetic stimulation and inhibition of hippocampal inputs to the AHN revealed that the HPC→AHN pathway not only mediates the contextual memory of predator threats but also controls the goal-directed escape flight by transmitting information about the surrounding environment. These results reveal a new mechanism for experience-dependent, top-down control of innate defensive behaviors.

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Poster

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Program #/Poster #: P659.06

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH 5R01MH106617
NARSAD /BBRF

Title: Prefrontal population coding guides contextual fear discrimination learning

Authors: *J. PASTORE[1], J. MAYER[1], T. BAILEY[1], J. I. SPIEGEL[1], J. STEINHAUSER[1], V. PAPALEXAKIS[2], M. CHROBAK[2], E. KORZUS[1];
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Abstract: The mechanistic theory of neural information storage speculates that neural representation activated during memory encoding is preferentially recruited during memory retrieval. Recent developments in network science are offering an excellent set of tools to analyze complex neural processes associated with learning and memory. Current studies use one-photon calcium imaging in freely behaving mice to assess large network dynamics and aim at identifying the neural representations of contextual fear memories and assess circuit dynamics underlying disambiguating safety and danger. Alterations of prefrontal network activity during fear modulation associated with context-dependent fear discrimination learning are evaluated via

real-time network activity assessment using miniaturized head-mounted microscopes followed by computational analysis of large-scale neuronal activity patterns. Head-mounted miniaturized microscopes are capable of measuring calcium transients reflecting neuronal activity from more than thousands of neurons in freely behaving mice. To evaluate large-scale circuit dynamics associated with fear discrimination learning, the calcium-sensitive fluorescent protein GCaMP6f is expressed in the prelimbic subdivision (PL) of the mPFC followed by assessment of real-time prefrontal network changes in response to dangerous and safe context stimuli across the behavioral testing. Our data indicate that PL local network is required for safety learning. To better understand these large and complex network dynamics, we use an unsupervised Tensor Decomposition in order to track neuronal populations involved in network learning across the temporal component of the behavioral sessions and across multiple days of stimuli (context) presentation. In addition, using the spike data of the calcium traces we constructed a new graph-theory-based computational model to better understand the overall network dynamics, population coding, and predictive properties of neural interactions. Our computation model revealed discrete alteration of global parameters of the large-scale network underlying safety learning and explains how these measures relate to the neural information-theoretic measure of predictive analysis. Understanding how fear memories are encoded and kept resistant to confusion is clinically relevant as fear memory overgeneralization is a hallmark of phobias, PTSD, and generalized anxiety disorder.

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Poster

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Program #/Poster #: P659.07

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH Grant MH115947
NSF Grant DGE144083

Title: Infralimbic prefrontal cortex neural activity associated with diurnal rhythms in auditory conditioned fear extinction in male rats

Authors: *M. J. HARTSOCK, M. J. NAVARRO, C. T. LEVY, M. P. SADDORIS, R. L. SPENCER;
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Abstract: Neurobiological mechanisms of memory are pervasively regulated by the circadian system. Individuals with post-traumatic stress disorder and related conditions show impairments

in fear extinction, a memory process by which a subject learns that cues that once predicted danger no longer do. Understanding how the circadian system regulates extinction memory may point to new approaches for optimizing extinction-based therapies in the clinic. Using rats, we have previously demonstrated that diurnal rhythms in fear extinction recall require normal circadian clock gene expression in the infralimbic prefrontal cortex (IL) (Woodruff et al. 2018, eNeuro). How the IL clock coordinates diurnal rhythms in extinction recall remains unknown. Here, we assessed IL neural activity during fear extinction at different times of day in adult male Sprague-Dawley rats maintained on a 12h:12h light-dark cycle. First, we bilaterally implanted 8-channel electrode arrays in the IL. Then, at either ZT4 (inactive phase) or ZT16 (active phase), rats underwent fear conditioning (Day 1: 3 tone-shock pairings in Context A), extinction learning (Day 2: 15 tone presentations in Context B), extinction recall (Day 3: 15 tone presentations in Context B), and fear relapse (Day 7: 15 tone presentations in Context B). We recorded neural activity in the IL on Day 2, Day 3, and Day 7 and subsequently time-locked freezing behavior to neural recordings by manually placing freeze onset and offset markers in videos captured with the Plexon Cineplex software. ZT4 and ZT16 rats showed comparable freezing behavior during the first three sessions, but ZT16 rats froze significantly less during the fear relapse session on Day 7. Our initial analyses of the neural data show changes in single-unit activity and local field potential power corresponding to tone onset, tone offset, freeze onset, and freeze offset. In our ongoing work, we will examine burst firing, identify putative excitatory versus inhibitory neurons, track isolated units and populations within and between sessions, and characterize potential time-of-day differences in neural activity.

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Poster

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Program #/Poster #: P659.08

Topic: G.01. Fear and Aversive Learning and Memory

Support: MH122023

Title: Nmda receptors in signal transmission: a computational perspective

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Abstract: NMDA receptors in signal transmission: a computational perspective
Greg Glickert[1], Benjamin Latimer[1], Pankaj Sah[2], and Satish S. Nair[1]

1 – Dept. of Electrical Engineering and Computer Science, Univ. of Missouri, Columbia, MO USA2 – Queensland Brain Institute, University of Queensland, Brisbane, Queensland AU
Hebbian, N-methyl-D-aspartate (NMDA) receptor-dependent long-term potentiation (LTP) is believed to underpin many forms of learning and memory. The Hebbian model of fear conditioning posits that repeated pairing of a conditioned stimulus (CS) with an aversive unconditioned stimulus (US) leads to LTP of synapses carrying CS information to the amygdala, and activation of these enhanced synapses evokes the conditioned response (CR). Fear learning and the associated synaptic plasticity has been shown to involve NMDA receptors. However, it is unclear whether in addition to facilitating potentiation of the CS pathway NMDA receptors also have a role in signal transmission itself. In particular, the role of these effects in fear discrimination remains to be explored. An interesting possibility is that intrinsic circuits may play an important role in engendering such plasticity, including via a recently discovered reverberatory circuit involving chandelier neurons in the amygdala. We analyze single unit recordings from the rodent basolateral amygdala during fear conditioning that show application of (7)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), an NMDA antagonist, shifted the excitation-inhibition balance toward inhibition during auditory stimulus presentation. CPP application impaired recruitment of putative inhibitory neurons during tone presentation. In this computo-experimental model, we construct a large-scale biophysically realistic network model to show how the dynamics of NMDA receptors facilitates both intrinsic neural activity and long-term potentiation in the basolateral amygdala during fear discrimination.

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Poster

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Topic: G.01. Fear and Aversive Learning and Memory

Support: NIMH Grant 1R01MH116203

Title: A thalamo-amygdalar CGRP circuit encoding aversive information

Authors: *S. KANG, S. LIU, M. YE, D.-I. KIM, J. KIM, S. HAN;
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Abstract: The perception of noxious stimuli motivates animals to perform immediate avoidance behaviors and to form aversive memories so that tissue-damaging stimuli can be avoided in the future. Thus, electrical footshock is often used as the unconditioned stimulus (US) in the classical associative threat learning paradigm. The amygdala plays a key role in this type of aversive learning. However, neural pathways by which nociceptive signals are conveyed to the

amygdala are not fully understood. Here, we identify that neurons expressing calcitonin gene-related peptide (CGRP) in the thalamus relay nociceptive information from the spinal cord to the lateral amygdala (LA). Using cutting-edge circuit dissection tools, such as fiber photometry, optogenetics, chemogenetics, and viral circuit tracing, we show that these neurons are activated by various nociceptive stimuli and form the spino-thalamo-amygdaloid pathway that is necessary and sufficient for affective-motivational pain perception. Furthermore, we find that this pathway is critical for conveying the US during the Pavlovian threat learning. The discovery of the cell-type-specific US pathway that transmits noxious inputs to the LA during associative threat learning provides critical circuit-based insights into developing therapeutic interventions for pain and threat-related disorders.

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Poster

P659. Threat/Fear: Neural Circuits IV

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Topic: G.01. Fear and Aversive Learning and Memory

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Brain and Behavior Research Foundation NARSAD Young Investigator Award to HAT
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Title: Medial prefrontal cortical dynorphin-containing neurons constrain emotional memories

Authors: *H. WANG[1], H. YARUR CASTILLO[1], R. FLORES GARCIA[1], N. LOOMBA[1], S. CASELLO[1], Q. WANG[1], G. OR[2], K. MAHE[2], L. TIAN[2], H. A. TEJEDA[1];
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Abstract: The medial prefrontal cortex (mPFC) is implicated in modulation of stress and anxiety. Dysfunction of mPFC circuitry is associated with several common symptoms in neuropsychiatric disorders, including cognitive deficits, anhedonia, and anxiety. The dynorphin (DYN) / kappa-opioid receptor (KOR) system has been implicated in mediating stress-induced adaptive and mal-adaptive behavior and is highly expressed within mPFC. However, our understanding of how the DYN / KOR system is embedded in mPFC networks and regulates circuit function is extremely limited. Here, by utilizing *in situ* hybridization we found that prodynorphin (PDyn) mRNA is expressed in subpopulation of mPFC glutamatergic and GABAergic somatostatin (SST) neurons. We then utilized *in-vivo* fiber photometry and single

cell imaging to demonstrate that mPFC DYN-expressing neurons are recruited during processing of threats and during retrieval of these memories. mPFC DYN neurons are not only activated by the threat itself but also tuned to respond to the cues that predict the threat upon associative learning. By using intersectional transgenic and viral approaches, we examined the activity of glutamatergic DYN neurons and GABAergic SST DYN neurons. We demonstrated that distinct DYN-containing cell types differentially encode threats and the cues that predict them. Moreover, we used a novel, fluorescence-based KOR sensor to probe the *in-vivo* dynamics of DYN / KOR transmission during fear conditioning. Similar to the activity of PDyn neurons, the KOR sensor signal is increased by threats and the cues that predict them, while responses to on-going threats are modulated in a dynamic manner. These results demonstrate the involvement of local DYN / KOR signaling during the Pavlovian threat conditioning. Finally, we demonstrated that manipulating DYN / KOR signaling via viral-mediated knockdown of PDyn expression using small hairpin RNA (shRNA) modifies Pavlovian threat conditioning. We find that PDyn knock-down mice exhibit enhanced freezing to threat-associated cues compared to control group during Pavlovian threat conditioning, without impacting expression of long-term memories or their extinction. In summary, we address the anatomical organization of DYN / KOR system in mPFC and demonstrate the role of mPFC DYN neurons and DYN / KOR signaling in formation of emotional memories. As dysregulation of mPFC circuits and DYN / KOR signaling therein have been implicated in various neuropsychiatric disorders, these results may provide a framework to inform the development of therapeutic targets focused on these systems.

Disclosures: H. Wang: None. **H. Yarur Castillo:** None. **R. Flores Garcia:** None. **N. Loomba:** None. **S. Casello:** None. **Q. Wang:** None. **G. Or:** None. **K. Mahe:** None. **L. Tian:** None. **H.A. Tejeda:** None.

Poster

P661. Fear Memory: Cellular and Molecular Mechanisms

Location: Virtual Only

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Program #/Poster #: P661.01

Topic: G.01. Fear and Aversive Learning and Memory

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MD007579 (B.R.A.I.N and M.A.G.I.C Core)
NIH-NIGMS 1R15 MH116345-01
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Title: Effects of single prolonged stress on the rat infralimbic cortex proteome

Authors: *Y. RIVERA-ESCOBALES[1], Y. CASTILLO-OCAMPO[2], Y. M. CANTRES-ROSARIO[3], A. E. RODRÍGUEZ-DE JESÚS[4], K. CARRASQUILLO-CARRIÓN[4], A. ROCHE-LIMA[4], C. SUAREZ-GÓMEZ[2], L. SAMBOLÍN-ESCOBALES[2], M. COLÓN-ROMERO[2], L. M.

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Abstract: One diagnostic criterion for post-traumatic stress disorder (PTSD) is exposure to a traumatic event. However, experiencing a traumatic event will not necessarily result in PTSD. The molecular mechanisms that underlie protection against traumatic sequels remain unclear. The single prolonged stress (SPS) is an animal model of PTSD recently develop that induces a PTSD-like behavior in rodents 7 days after exposure to three different stressors. This model consisting of restraint stress, forced swim test and ether-induced loss of consciousness has proven to induce behavioral and molecular characteristics similar to humans with PTSD. Still, similar to humans, rats exposed to SPS show variability in their behavior. Not all rats exposed to SPS end up developing a PTSD-like phenotype. It should be noted that although the prevalence of PTSD is twice in women than men, few studies include female rats in their research. The few studies including female rats suggest that they are not sensitive to the effects of SPS. Thus, in this study we aim to investigate the molecular mechanisms underlying the variability in behavior in SPS exposed animals. To this end, we exposed male and female Sprague Dawley rats (p60) to the SPS. Seven days after we analyzed anxiety-like behavior using the open field test and animals were trained in fear conditioning. Results showed no difference in anxiety like behavior between SPS animals or sex. Fear conditioning results showed variability in the ability to extinguish the fear memory. From a pool of 8 male and female SPS exposed animals' half of them presented impairment in fear memory extinction. The other half were able to recall the extinction memory. Classification of fear extinction deficits was determined based on the number of freezing percent the animals exhibited during the extinction training recall test. Animals with a freezing percent above 50 were considered to have impaired fear memory extinction, whereas animals with a freezing percent below 50 were considered to have extinguished the fear memory. Since the infralimbic cortex has been reported to have a role in fear memory extinction, we collected infralimbic cortex tissue for a proteomic analysis of membrane associated proteins of those animals with impaired or enhanced fear memory extinction. Proteomic analysis is undergoing, we expect our results to provide a better insight of the molecular mechanisms associated with fear memory extinction deficits. Our main goal is to identify potential therapeutic targets for pharmacological treatment of this disease. As well as to understand what factors might be providing protection to individuals individual whom to not develop this disease.

Disclosures: Y. Rivera-Escobales: None. **Y. Castillo-Ocampo:** None. **Y.M. Cantres-Rosario:** None. **A.E. Rodríguez-De Jesús:** None. **K. Carrasquillo-Carrión:** None. **A. Roche-Lima:** None. **C. Suarez-Gómez:** None. **L. Sambolín-Escobales:** None. **M. Colón-Romero:** None. **L.M. Meléndez-Aponte:** None. **J.T. Porter:** None.

Poster

P661. Fear Memory: Cellular and Molecular Mechanisms

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Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH-NIGMS 5R25GM082406 (PHSU RISE Graduate Training Program),
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NIH-NIGMS 1R15MH116345-01

Title: Reducing FKBP5 expression in the ventral hippocampus of adult male rats reduces conditioned fear

Authors: *N. IRIZARRY-MÉNDEZ, A. HERNANDEZ, M. COLON, J. T. PORTER;
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Abstract: Reducing FKBP5 expression in the ventral hippocampus of adult male rats reduces conditioned fear

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Post-traumatic stress disorder (PTSD) is a neuropsychiatric disorder that may develop after exposure to an overwhelming traumatic event, such as rape, war or an accident. Memories of traumatic events are reinforced by glucocorticoid signaling which can induce changes in ventral hippocampal (VH) neurons to regulate memory consolidation. The hippocampus is involved in both learning and memory processes and endocrine regulation, and its function is susceptible to disruption by stress. Excessive glucocorticoid signaling induced by stress is inhibited by FKBP5 protein, which decreases glucocorticoid receptor affinity for glucocorticoids. Human postmortem investigations imply that increased FKBP5 expression may play a role in psychiatric phenotypes. Also studies in rodents suggest that abnormal glucocorticoid signaling in the fear circuits may play a role in the development of PTSD-like phenotypes. Despite the association of FKBP5 with PTSD, it is unclear if altering FKBP5 expression contributes to the susceptibility or resiliency to PTSD. This study aims to examine if modulation of FKBP5 expression in the VH is sufficient to affect fear acquisition or extinction. We hypothesized that reducing FKBP5 protein expression in the VH would increase glucocorticoid signaling to enhance auditory fear memory and reduce extinction. To test this, an AAV5 vector expressing 4 different shRNAs that target FKBP5 and the fluorescent marker mCherry was infused into the VH to reduce FKBP5 levels in adult male rats. One month later, rats were exposed to auditory fear conditioning and extinction and then sacrificed for brain tissue collection. The infusion site and viral efficiency were verified with immunofluorescence labeling of mCherry and FKBP5. In contrast to our hypothesis, we found that reducing FKBP5 in VH decreased fear acquisition and facilitated fear extinction memory. Our findings suggest that people with lower the expression of hippocampal FKBP5 might acquire less fear during trauma exposure and show resiliency to PTSD.

Disclosures: N. Irizarry-Méndez: None. **A. Hernandez:** None. **M. Colon:** None. **J.T. Porter:** None.

Poster

P661. Fear Memory: Cellular and Molecular Mechanisms

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Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH Grant K99MH119320

Title: Bdnf bioavailability differentially impacts adolescent and adult safety learning

Authors: *H. C. MEYER, F. S. LEE;
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Abstract: A prevailing molecular framework thought to underlie mood and anxiety disorders is the neurotrophic hypothesis, which postulates that reductions in neurotrophic support can alter neural plasticity in key regions implicated in anxiety and fear responses. In line with this, to examine how the neurotrophin BDNF contributes to the pathophysiology of anxiety disorders our lab has utilized a knock-in mouse line carrying the human Val66Met polymorphism, which leads to decreased BDNF bioavailability. We have previously shown that adult mice expressing the Met allele recapitulate the phenotypic hallmarks of humans with this polymorphism, especially with regard to altered anxiety- and fear-related behaviors. While substantially less is known about the impact of BDNF signaling on the development of fear-related circuits, our lab has shown that genotypic differences observed in adulthood are not apparent for extinction in adolescent mice expressing either the Val or Met allele, although both groups exhibit blunted extinction learning. These findings indicate the need for novel methods of fear regulation that can be utilized both during adolescence and also during adulthood in populations with disrupted neurotrophic signaling. To address this, we examined the capacity for Val66Met mice and age-matched controls to utilize a conditioned safety signal to directly inhibit fear responding. Wild-type adolescent mice and adult mice of both genotypes were able to acquire and use (i.e., conditioned inhibition) a safety signal to inhibit fear. In contrast, adolescent mice expressing the Met allele exhibited impaired conditioned inhibition. Our data suggest a developmental dissociation in the impact of BDNF bioavailability on the neural circuitry underlying extinction learning and conditioned inhibition. Ongoing studies are investigating how the relative distribution of BDNF throughout fear regulation circuitry across development (focusing on the ventral hippocampus and medial prefrontal cortex) directly relates to fear expression profiles. In sum, this line of research will provide insight into both age- and genotype-informed treatments for anxiety disorders.

Disclosures: H.C. Meyer: None. **F.S. Lee:** None.

Poster

P661. Fear Memory: Cellular and Molecular Mechanisms

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Program #/Poster #: P661.04

Topic: G.01. Fear and Aversive Learning and Memory

Support: NSF GRFP

Title: Anatomical Connectivity of the Intercalated Cells of the Amygdala

Authors: *D. STERN[1], C. M. ROOT[2];
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Abstract: The Intercalated Cells of the Amygdala (ITCs) represent a unique, understudied subset of exclusively inhibitory neurons within the amygdala that are phylogenetically conserved and share developmental, morphological, and biochemical similarities with cells of the striatum. ITCs reside within the intermediate and external capsules surrounding the BLA and can be grouped into three separate nuclei that extend nearly the entire rostro-caudal extent of the amygdala: the main, caudal, and lateral nuclei. The transcription factor FoxP2 is a known genetic marker of the ITCs, and the FoxP2-cre mouse was recently used to refine our understanding of their role in fear conditioning, extinction, and recall. We used FoxP2-cre mice and small iontophoretic injections of cre dependent viruses to investigate the upstream and downstream connectivity of the three ITC nuclei. For downstream connectivity, we used a synaptophysin virus that expresses tdTomato cytoplasmically and a synaptophysin-GFP conjugate that shows putative synapses (n=18 mice, 9 male / 9 female, 3-9 months old). The three ITC nuclei project ipsilaterally in overlapping but distinct patterns to large areas within the basal forebrain, bed nucleus of the stria terminalis, and amygdala. The densest projections are sent to the basal forebrain, medial amygdala, cortical amygdala, and other ITC nuclei. Contrary to prior thinking, there are relatively sparse projections to the basolateral and central amygdalae. For upstream connectivity, we used a cre-dependent monosynaptic rabies approach to reveal neurons that project to the ITCs (n=18 mice, 9 male / 9 female, 3-9 months old). All presynaptic projections are ipsilateral and include dense clusters in the insular cortices, paraventricular thalamic nucleus, posteromedial cortical amygdala, entorhinal cortex, hippocampus, ethmoid nucleus, substantia nigra and posterior thalamic nuclei. Additionally, there are numerous but diffuse presynaptic neurons throughout the basal forebrain and amygdalar nuclei. Surprisingly, we see only a few presynaptic neurons within the BLA, and none in the prelimbic and infralimbic cortex, challenging prior work on the hypothesized network underlying fear conditioning, extinction, and recall. This work makes clear the comparative projections to and from the separate ITC

nuclei, demonstrates significantly more abundant projections than prior work, and suggests that the ITCs are well positioned to influence a broad range of amygdala and basal forebrain mediated behaviors by integrating information from a wider range of structures than previously known.

Disclosures: D. Stern: None. **C.M. Root:** None.

Poster

P661. Fear Memory: Cellular and Molecular Mechanisms

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Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P661.05

Topic: G.01. Fear and Aversive Learning and Memory

Title: Role of PP1 in conditioned inhibition related reductions in type B photoreceptor excitability

Authors: *J. B. ANDERSON[1,2], J. S. CAVALLO[1,2], J. FARLEY[1,2];
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Abstract: Explicitly unpaired (EU) presentations of light and rotation produce behavioral changes (e.g., increased phototaxis) in the sea snail Hermissenda crassicornis that satisfy the behavioral criteria for conditioned inhibition (CI) learning. This is accompanied by decreased excitability of type B photoreceptors (B cells) due to corresponding increases in potassium (K^+) currents. We've previously shown that protein phosphatase 1 (PP1) inhibitors (e.g., Calyculin A): 1) depolarize Untrained B cells and reduce somatic K^+ currents, partially mimicking the effects of associative conditioning (light - rotation pairings), 2) contribute to the excitability (spike frequency) decreases produced by extinction training (a form of learning that overlaps mechanistically with EU conditioning). Conversely, injection of catalytically-active PP1 (caPP1) into Untrained B cells partially mimicked the spike frequency declines produced by extinction training, and occluded further reductions in spiking by additional extinction training. Thus, we hypothesized that PP1 activity may also contribute to EU-training effects on B cells. To test this, we measured B cell photoresponses from Untrained and EU-trained animals following treatment with caPP1. B cells showed marked reductions in steady state generator potentials (SSGPs) and spike frequencies over the course of five 30-sec test lights, mimicking EU training. Prior EU training the effects of caPP1 injection. Additional measurements showed that caPP1 increased both the transient (I_A) and delayed ($I_{K\text{-}Ca}$) components of K^+ current, previously shown to mediate the EU-related decrease in B cell excitability. We next injected B cells from Untrained and EU-trained animals with the core peptide fragment of the PP1 regulatory G subunit (GM), which acts as a competitive inhibitor of PP1. Iontophoresis of 25uM GM increased B cell SSGPs from untrained (14.3%, n.s.) and EU-trained (45.7%; $p = 0.0332$) animals. Whereas EU training resulted in a 41.6% reduction in B cell SSGP ($p < 0.001$), SSGPs of GM treated B cells were not

significantly different from untreated B cells of Untrained animals. Thus, GM treatment abolished the EU-related decrease in B cell excitability. Our results suggest that: 1) upregulation of PP1 activity is an important component of the EU-mediated decrease in B cell SSGP, 2) PP1 is constitutively active in B cells of Untrained animals. These data indicate that EU training increases PP1 activity in B cells, decreasing excitability through effects on downstream K^+ currents.

Disclosures: J.B. Anderson: None. **J.S. Cavallo:** None. **J. Farley:** None.

Poster

P661. Fear Memory: Cellular and Molecular Mechanisms

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Program #/Poster #: P661.06

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH Grant 2TR01ActMH107435Project06

Title: Endocannabinoid Regulation of Predator Odor Avoidance

Authors: *V. KONDEV[1], A. MORGAN[1], **M. NAJEED**[1], S. PATEL[2];
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Abstract: Anxiety disorders are the most common mental illness in the United States and symptoms characteristic of these disorders include hyperarousal, excess fear, and enhanced avoidance even in the absence of immediate threat. The endocannabinoid (eCB) system regulates fear responsivity and stress adaptivity and thus has been identified as an emerging therapeutic target to treat the underlying pathology. Importantly, eCB signaling has been found to potently modulate avoidance behaviors, especially under conditions of high environmental aversiveness. However, the role that the eCB system plays in mediating avoidance during an innate predator odor stress exposure has not been thoroughly investigated. Here, we have determined a reliable, dose-dependent protocol that induces avoidance in both CD-1 and C57 strains of mice to 2-methylthiazoline (2-MT), a synthetic analog of the highly volatile molecule found in fox odor. Given that the eCB system plays a prominent role in approach-avoidance conflicts, we demonstrate that 2-arachinoylglycerol (2-AG), but not anandamide, regulates avoidance to this innate stressor. Specifically, pharmacologically enhancing 2-AG levels with treatment with the MAG lipase inhibitor, JZL-184, mitigates avoidance of the odor source. This data sheds light onto the therapeutic potential of eCB modulators in treating the hypervigilant state associated with pathological fear and anxiety.

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Poster

P661. Fear Memory: Cellular and Molecular Mechanisms

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Topic: G.01. Fear and Aversive Learning and Memory

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Title: Cued fear conditioning inhibits neurons of the bed nucleus of the stria terminalis (BNST) to the central amygdala

Authors: *V. OLIVERA-PASILIO[1], J. DABROWSKA[2];
[1]Rosalind Franklin Univ. Sch. of Grad. and Postdoctoral Studies, North Chicago, IL; [2]Rosalind Franklin Univ., North Chicago, IL

Abstract: The dorsolateral bed nucleus of the stria terminalis ($BNST_{DL}$) contains GABA-ergic neurons co-expressing various neuropeptides. $BNST_{DL}$ sends inhibitory projection to the central amygdala (CeA), and activation of this projection increases anxiety-like behavior. However, based on the dominant role of the CeA in cued fear acquisition, the inhibitory BNST-CeA neurons might also suppress cued fear. This is also supported by our recent results demonstrating that activation of oxytocin receptors (OTR) in the $BNST_{DL}$ inhibits BNST-CeA neurons and facilitates cued fear. To determine the phenotype of the BNST-CeA neurons, adult male Sprague-Dawley rats (SD; n=4) were injected with retrograde pENN.AAV.hSyn.GFP-Cre into the CeA. BNST-CeA neurons expressing eGFP were co-labeled with antibodies against protein kinase C δ (PKCδ), striatal-enriched protein tyrosine phosphatase, calbindin, somatostatin, and cholecystokinin 8. BNST-CeA neurons co-express all these markers at specific sub-nuclei of the $BNST_{DL}$, suggesting diverse content of the projection. As the majority of the BNST-CeA neurons in the oval nucleus of the $BNST_{DL}$ co-expressed PKCδ, we next determined if PKCδ neurons were recruited during fear conditioning. Male SD rats (n=18) were subjected to 10 foot shocks (un-signaled or signaled by a discrete cue) or were placed in chambers as controls; 45 min after injection of non-peptide OTR antagonist (L-368,899, 5 mg/kg) or vehicle. Neurons expressing PKCδ or cFos and co-expressing both markers were counted in the $BNST_{DL}$ using ImageJ. Here, cued fear conditioning tended to reduce the percentage of neurons co-expressing PKCδ+cFos per total number of cFos neurons, an effect reversed by L-368,899 (treatment $F(1,30)=3.593$ $p=0.0677$, interaction between fear and treatment $F(2,30)=3.302$ $p=0.0506$), suggesting that cued fear conditioning inhibits $BNST_{DL}$-PKCδ neurons via OTR-dependent mechanism. To determine the recruitment of BNST-CeA neurons in fear acquisition, male SD rats (n=9) were injected with the retrograde AAV-eGFP into the CeA and 2 weeks later were subjected to 10 predictable foot shocks (paired with cue as above), or 10 un-paired foot shocks, or 10 visual cues alone. Exposure to foot shocks paired with cue reduced the percentage of

BNST-CeA-eGFP neurons expressing cFos per total of eGFP neurons (fear effect F (2, 26) = 5.696 p=0.0089, in comparison to control, p<0.01). Overall, our results suggest that cued fear conditioning inhibits BNST-CeA neurons via OTR-dependent fashion. This might be an important mechanism by which OTR attenuates the inhibitory influence of the BNST over the CeA and facilitates cued fear.

Disclosures: V. Olivera-Pasilio: None. **J. Dabrowska:** None.

Poster

P661. Fear Memory: Cellular and Molecular Mechanisms

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Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH NIDA R01DA037327
NIH NIDA F30DA048597

Title: The role of serotonin 5-HT2C receptors at the RMTg in cocaine conditioned avoidance

Authors: *Y. CHAO[1], J. PARILLA CARRERO[1], M. EID[1], P. J. VENTO[2], T. C. JHOU[1]; [1]Med. Univ. of South Carolina, Charleston, SC; [2]Univ. of South Carolina, Columbia, SC

Abstract: Relatively little is known about the processes that underlie cocaine's aversive effects. Aversive responses to cocaine are significant because they may modulate both the acquisition of drug-seeking, and later behaviors such as relapse in experienced animals, and may underlie individual differences in susceptibility to cocaine addiction. Prior work from our lab showed that activation of the rostromedial tegmental nucleus (RMTg) by cocaine critically drives cocaine conditioned avoidance. While some of this cocaine-induced RMTg activation is due to glutamatergic inputs from the lateral habenula, a majority arises from unknown mechanisms. Using RNAseq we made the unexpected discovery that the RMTg expresses robust and enriched levels of mRNA for the serotonin 2C receptor (gene name *htr2c*) relative to surrounding regions. Because cocaine binds to both serotonin and dopamine uptake transporters, we postulate that 5-HT_{2C}R activation at the RMTg might be a novel mechanism by which cocaine activates the RMTg and drives aversive response. Using in situ hybridization technique, we find 80% of RMTg neurons are *htr2c* positive. Most of these *htr2c* positive neurons express putative markers of RMTg neurons *gad1* and *foxp1*, and a significant proportion of them responds with elevated *cfos* to a diverse array of aversive stimuli. Next, using behavior paradigms that have been established to be sensitive to the aversive properties of cocaine, we find injection of Ro60-0175, a specific 5-HT_{2C}R agonist, is sufficient to induce conditioned place aversion and enhance anxiety-like behavior, while both intra-RMTg injection of SB-242084, a specific 5-HT_{2C}R antagonist, and RMTg site-specific shRNA knockdown of the *htr2c* gene, reduce cocaine-

induced anxiety like behavior, block cocaine conditioned place aversion, and attenuate cocaine conditioned avoidance. Using Sprague Dawley rats that have been trained to develop divergent low versus high avoidance response to cocaine, we find knockdown of *htr2c* at the RMTg increases cocaine self-administration and reinstatement in high-avoider but not low-avoider animals. Further, using slice electrophysiology techniques, we find both cocaine and 5-HT_{2C}R agonist induce depolarization of RMTg neurons that can be blocked by specific antagonist and *htr2c* specific shRNA. These evidences support an important role of RMTg 5-HT_{2C}R in the aversive effects of cocaine, and the notion that aversive effects of cocaine modulate subsequent relapse drug seeking. The current studies may identify a valid target for treating cocaine's aversive effects and relapse drug seeking.

Disclosures: Y. Chao: None. **J. Parilla Carrero:** None. **M. Eid:** None. **P.J. Vento:** None. **T.C. Jhou:** None.

Poster

P661. Fear Memory: Cellular and Molecular Mechanisms

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Program #/Poster #: P661.09

Topic: G.01. Fear and Aversive Learning and Memory

Support: CAPES

Title: The interplay between vanilloid and endocannabinoid signalling in the hippocampus is modulated by the intensity of the contextual fear conditioning

Authors: *L. P. IGLESIAS[1], H. B. FERNANDES[2], A. S. DE MIRANDA[2], C. A. SORGI[3], F. A. MOREIRA[1];
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Abstract: The TRPV1 channel has been proposed as potential target for the modulation of fear memories. TRPV1 is activated by anandamide (AEA), which also binds to the CB1 receptor. It remains unclear in which circumstances anandamide and TRPV1 signalling is recruited in contextual fear conditioning (CFC). Here, we tested the hypothesis that TRPV1 blockers prevent CFC retrieval under specific intensities of aversive stimuli. We also investigated the underlying mechanisms. Male C57BL/6J mice (n=5-8/group) were submitted to different shock intensities in the CFC paradigm and received local administration of SB366791 (SB, 3nmol), a TRPV1 blocker, into the dorsal hippocampus (dHPC) before fear memory retrieval. The hippocampus was removed for PCR quantification of selected genes and for mass spectrometry analysis of AEA levels. To study the involvement of CB1 and AEA, a subeffective dose of AM251, a CB1 antagonist, was administered prior to SB. Double immunofluorescence staining was used to

evaluate TRPV1-CB1 co-localization. The data was analysed by ANOVA followed by Bonferroni post-hoc test. SB prevented CFC retrieval in animals conditioned under aversive stimuli of moderate (MI) and high (HI), but not low intensity (LI). The genes related to neural plasticity, Arc, Zif and Trkb, were upregulated after SB treatment in the MI but not in the HI group. In addition, the animals treated with SB in the MI, but not in the HI protocol, were resistant to CFC reinstatement. The local levels of AEA positively correlated with freezing levels. Finally, we found that TRPV1 and CB1 are co-localized in the dHPC and that AM251 prevented SB effects. In conclusion, dHPC TRPV1 channels are recruited as a function of the aversive stimulus intensity. At low-intensity aversive stimulus, AEA levels may not be sufficient to recruit TRPV1 channels. However, at medium and high intensity aversive stimuli, AEA levels are increased to facilitate TRPV1-mediated CFC. Under this circumstance, TRPV1 blockade prevents fear retrieval by shifting AEA signalling to CB1-mediated mechanisms and possibly recruiting Arc, Zif and Trkb transcription. The recruitment of this pathway was associated with reinstatement resistance, which was not observed at the highest intensity. Acknowledgement: CAPES

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Poster

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Program #/Poster #: P661.10

Topic: G.01. Fear and Aversive Learning and Memory

Title: Astrocytic GluN2C-containing NMDA receptors in the basolateral amygdala facilitate fear extinction

Authors: *G. P. SHELKAR, J. LIU, S. M. DRAVID;
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Abstract: Abstract: Fear extinction is a learned inhibitory response to a conditioned fear when the initially noxious conditioned responses are no longer associated with aversive outcomes, and it is an important model of cognitive-behavioral therapy for human anxiety disorders. The basolateral amygdala (BLA) is one of the brain nuclei that play an essential role in fear extinction. Recently we have identified predominant expression of GluN2C-containing NMDA receptors in astrocytes in BLA; however, their functional roles remain poorly understood. Herein, we took advantage of the novel highly potentGluN1/GluN2C NMDA receptors selective co-agonist, AICP, to assess the functional role of GluN2C subunits in fear extinction. We found that intracerebroventricular injection of AICP produced robust facilitation of fear extinction in WT mice. However, this effect was absent in GluN2C KO mice. Further, local injection of AICP

in BLAfacilitated fear extinction in WT but not in GluN2C KO mice, suggesting that AICPproduces its effect via GluN2C-containing receptors in the BLA. Knowing the astrocytic expression of GluN2C subunit in the BLA, we used conditional knockout strategy to delete obligatory GluN1 subunits from astrocytes. Intra-BLA injection of AICP did not facilitate fear extinction in mice with conditional deletion of obligatory GluN1 subunit from astrocytes. Notably, chemogenetic activation of astrocytes in the BLA facilitated fear extinction. In electrophysiological studies, bath application of AICP facilitates excitatory neurotransmission in the BLA via a presynaptic GluN2C-dependent mechanism. Furthermore, in immunohistochemical analyses, AICP treatment showed significant upregulation of GluA1 AMPA receptors and their colocalization with PSD 95. Together, our results showed an essential role of astrocytic GluN2C-containing NMDA receptors in the extinction of conditioned fear memory and propose that further development of superagonists of GluN2C-containing receptors may offer a novel therapy for PTSD and anxiety-like disorders.

Disclosures: G.P. Shelkar: None. **J. Liu:** None. **S.M. Dravid:** None.

Poster

P705. Anxiety: Pre-Clinical Models

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P705.01

Topic: G.06. Anxiety Disorders

Support: Artelo Biosciences
MITACS Canada

Title: A novel FABP5 inhibitor modulates anxiety-related signaling patterns in the basolateral amygdala-prefrontal cortical-hippocampal circuitry

Authors: *M. J. JONES[1], T. C. UZUNESER[1], M. KACZOCHA[2], I. OJIMA[2], S. R. LAVIOLETTE[1];
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Abstract: Anxiety disorders are a class of disorders characterized by pathological anxiety and stress symptoms, impacting one in three people throughout their lifetime. Currently available medications for the treatment of anxiety-related symptoms are associated with significant side-effects such as dependence, withdrawal, and cognitive problems. This calls for the development of alternative pharmacotherapies. The endocannabinoid (eCB) signaling system is critically involved in anxiety-related neural processing. We have previously reported that CB1 receptor transmission within the basolateral amygdala (BLA) can strongly modulate emotional sensory information salience and associative memory formation via modulation of neuronal activity patterns in the prefrontal cortex (PFC) and ventral hippocampus (vHIPP)[1]. Anandamide, a key

eCB ligand, has limited ability for diffusion, causing transport for degradation to be largely reliant on Fatty Acid Binding Proteins (FABPs) — with the FABP5 subtype displaying one of the highest affinities. We hypothesized that FABP5 inhibition with a novel FABP5 pharmacological inhibitor (SBFI-103) in the BLA would increase localized anandamide levels leading to suppression of anxiety-related activation of BLA neurons and functional outputs to neuronal populations in the PFC and vHIPP, resulting in anxiolytic outcomes. Using *in vivo* single-unit recordings in rats, we found that FABP5 inhibition in the BLA produced a significant increase in the spontaneous firing and bursting rate of PFC neurons, accompanied by an increase in gamma oscillatory activity, both of which are associated with anxiolytic and cognitive processing phenotypes. Furthermore, we observed significant changes in vHIPP neuronal activity dynamics following intra-BLA FABP5 inhibition, most notably associated with increased theta oscillatory activity, suggesting potential modulation of fear memory processing. Remarkably, these effects in the PFC and vHIPP were blocked when the FABP5 inhibitor was co-administered with a selective CB2 receptor antagonist, suggesting a novel functional role for CB2 receptor transmission in these phenomena. Future studies will investigate the behavioural effects of FABP5 inhibitors in the BLA on anxiety-related behaviours. Together, these findings demonstrate a novel, FABP5-dependent mechanism directly in the BLA regulating anxiety-like phenotypes and identify a novel pharmacotherapeutic role for this FABP5 inhibitor within anxiety-related neural circuitry.
1. Biol. Psychiatry. 80(3):216-25; J. Neurosci. 31(14):5300-12; Cereb Cortex. 20(6):1486-96

Disclosures: M.J. Jones: None. **T.C. Uzuneser:** None. **M. Kaczocha:** None. **I. Ojima:** None. **S.R. Laviolette:** None.

Poster

P705. Anxiety: Pre-Clinical Models

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Program #/Poster #: P705.02

Topic: G.06. Anxiety Disorders

Title: Induction and sensitivity to apprehensive expectation, a model of chronic trait anxiety in mice.

Authors: *D. W. CRAWFORD, K. M. MCKEEN, H. M. PATEL, L. D. MATZEL;
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Abstract: The use of animal models to assess characteristics of human psychological disorders is predicated on the presumption that the model is analogous to the human condition in symptomology, duration, differentiation from disparate disorders, etiology, and sensitivity to known treatments. Unlike those of some notable other disorders (e.g., depression), current rodent models of anxiety fail to satisfy these requirements, instead serving as simple measurements of

variations in the expression of an “anxious” response when the animal is placed in a state of temporary conflict. While these current models (e.g., the elevated plus maze) *are* sensitive to anxiolytic drugs, they fail to induce a chronic state of behavior, i.e., a trait, and do not differentiate between related states (e.g., fear, acute stress). Relatedly, common models make little contact with theories of the etiology of anxiety. In the present study, we propose a novel operantly-induced model of chronic anxiety in mice aimed at overcoming these pervasive limitations. Here, genetically heterogeneous mice were exposed to environmental conditions which induce a state of “apprehensive expectation,” a fundamental characteristic of human anxiety disorders as described by the DSM-V. Mice were subjected to a 15 day treatment of unpredictably interspersed food reinforcement *or* mild shock after entering the terminal goal area of a straight alley. A one-way ANOVA with planned comparisons revealed that mice exposed to this treatment exhibited significantly elevated levels of anxiety-like behaviors compared to mice that received either consistent food or shock in the maze ($t = 2.41$, $p = .02$). No significant differences in anxiety-like behaviors were observed between groups prior to these treatments ($F = .04$, $p = .96$). These results suggest this novel apprehensive expectation manipulation had induced a chronic anxiety-like trait in the mice exposed to the emotionally conflicting outcomes (i.e., food or shock). This anxiety-like trait may have an etiology more aligned with that of the human disorder and is empirically separable from simple fear (i.e., shock-only control). Furthermore, there was a significant correlation between the pre- and post-treatment measures of anxiety-like behaviors in the emotional conflict group ($r = .45$, $p = .04$), suggesting that pre-treatment measures of state anxiety capture a predisposition for induction of trait anxiety. Future studies are underway to assess the induction of this model using a classical conditioning paradigm, as well as to test the model’s susceptibility to anxiolytic drug intervention to ensure the model retains the predictive validity expressed by current models.

Disclosures: D.W. Crawford: None. **K.M. McKeen:** None. **H.M. Patel:** None. **L.D. Matzel:** None.

Poster

P705. Anxiety: Pre-Clinical Models

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Program #/Poster #: P705.03

Topic: G.06. Anxiety Disorders

Support: NIH R01 MH115027

Title: Encoding of anxiety learning and diazepam treatment by rodent prefrontal cortex and VTA neurons

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[1]Behavioral Neurosci., Oregon Hlth. & Sci. Univ., Portland, OR; [2]Neurosci., HHMI Janelia Res. Campus, Ashburn, VA

Abstract: Background: Learning that routine reward motivated actions are associated with the risk of an aversive event is critical for development and expression of anxiety. To model this real-life scenario, we designed a task where instrumental actions taken toward obtaining a reward were probabilistically punished with a mild footshock. We addressed the outstanding questions of whether the ventral tegmental area (VTA) and rodent medial prefrontal cortex (mPFC) adapt to learning of punishment risk in correlation with behavior, and how diazepam influences these neural responses.
Methods: Fiber photometry was used to measure pan-neural activity in mPFC and VTA in adult male and female rats. This method allowed us to quantify mPFC and VTA neural response to the shock itself as well as during cue and action encoding epochs which is critical to distinguish how neural responses adapt to receiving versus expecting an aversive experience.
Results: We find that selective suppression of male and female behavior developed after 2-3 sessions of exposure to punishment risk. This change, or learning, was accompanied by a reduction of phasic neural activity in mPFC and an upward shift in VTA activity, primarily during the peri-action period. Footshock produced a robust activation of neural activity in mPFC and VTA that remained consistent in magnitude after learning. Diazepam did not change the phasic neural response of mPFC or VTA to the shock nor did it influence mPFC responses to task events. Diazepam did, however, significantly and selectively produce a peri-action ramping response in the VTA and enhanced correlative VTA-mPFC activity.
Conclusions: These findings characterize the adaptive neural responses of mPFC and VTA during the learning of anxiogenic contingencies that are independent from the aversive experience itself. They also identify peri-action ramping of VTA activity as a potential marker for anxiolytic properties of diazepam. This ramping signal has been linked to preparatory attention and goal driven states, potentiation of this response may lead to attentional disengagement from harm probability and provide a mechanism for how diazepam influences action selection under anxiety.

Disclosures: D.S. Jacobs: None. **M.C. Allen:** None. **M.L. Kielhold:** None. **J. Park:** None. **B. Moghaddam:** None.

Poster

P705. Anxiety: Pre-Clinical Models

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Topic: G.06. Anxiety Disorders

Support: NIAAA Grant R21AA027873-01A1

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Title: Moderate prenatal alcohol exposure on gestational day 12 produces sex-specific social impairments and alters prelimbic cortex physiology

Authors: *K. R. PRZYBYSZ[1], E. I. VARLINSKAYA[2], M. R. DIAZ[2];
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Abstract: Prenatal alcohol exposure (PAE) is capable of producing many psychological, cognitive, and behavioral deficits, and social impairment is the most common behavioral problem associated with PAE. Rodent models examining PAE have identified gestational day (G) 12 as a critical developmental point for producing social impairments across the lifespan. Importantly, in the human population, low-moderate PAE (mPAE) is more prevalent than binge-level PAE; despite this, whether mPAE on G12 can also produce similar social impairments as binge-level exposure, and the potential neuroadaptations that may underlie them, has not been examined. Two brain regions that modulate social behaviors are the basolateral amygdala (BLA) and prelimbic cortex (PL). The PL specifically is involved in producing negative social behaviors, such as social avoidance, and stimulation of the neural projection from the BLA to the PL (BLA-PL) decreases social interaction. However, whether these structures are altered by PAE, and whether they play a role in PAE-induced social impairments, is unknown. To examine these questions, we exposed pregnant Sprague-Dawley rats to vaporized ethanol or room air for 6 hours on G12 (blood ethanol concentration: 60-90 mg/dL) and tested their male and female offspring on the modified social interaction paradigm. This resulted in sex-specific social impairments that lasted into adulthood. Specifically, we found that both mPAE-exposed males and females exhibited reduced social investigation, while only males had reduced social preference. We then performed whole cell patch clamp electrophysiology experiments on PL pyramidal cells from a separate set of adult male and female offspring and found that, while there was no effect of mPAE on basal glutamate transmission, cells from mPAE-exposed males and females had significantly reduced intrinsic excitability. Finally, to examine the role of the BLA-PL pathway in mPAE-induced social deficits, we used a chemogenetic approach to inhibit the BLA-PL during social behavior testing with the hypothesis that inhibiting the BLA-PL would recover normal social behavior in mPAE offspring. Our preliminary data suggest that this projection, and its role in modulating social behavior, are potentially altered following mPAE in a sex-dependent manner. Taken together, we have identified multiple neural mechanisms that may play a role in PAE-induced social impairments, as this work is among the first to examine mPAE-induced changes to PL physiology and to probe neural circuits in the context of PAE. This will be highly valuable in furthering our understanding of the neuroadaptations underlying PAE-induced social dysfunction.

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Poster

P705. Anxiety: Pre-Clinical Models

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Topic: G.06. Anxiety Disorders

Support: Boehringer Ingelheim Fonds PhD fellowship
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Title: Cell-type-specific dopamine signaling in ventral hippocampus tracks anxiety-related behavior

Authors: *A. GODINO[1], M. SALERY[1], A. M. MINIER-TORIBIO[1], J. F. FULLARD[2], E. M. PARISE[1], F. J. MARTÍNEZ-RIVERA[1], C. MOREL[1], S. E. MONTGOMERY[1], M.-H. HAN[1], P. ROUSSOS[2], E. J. NESTLER[1];
[1]Nash Family Dept. of Neurosci. & Friedman Brain Inst., [2]Dept. of Psychiatry & Friedman Brain Inst., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Anxiety is an emotional response that, under normal conditions, promotes adaptive avoidance behaviors, but, when inappropriate to the level of threat, can contribute to several psychiatric disorders. Despite accumulating evidence for a role of both the ventral hippocampus (vHipp) and the mesocorticolimbic dopamine system in encoding anxiety-relevant information, surprisingly little is known about how dopamine signaling selectively affects vHipp representations of emotionally-salient stimuli to guide innate approach/avoidance behaviors. To address this knowledge gap, we here study dopamine release and dopaminoceptive neurons in mouse vHipp – which can be segregated based on their expression of either the dopamine D1 or D2 receptor – as performed routinely for striatum – to delineate a model of dopamine action in vHipp. At the histological level, D1- and D2-expressing cells exhibit a precise topographical organization across vHipp subfields. We further dissected their transcriptional profiles and cell-type identity using RNA-sequencing of single, sorted nuclei from D1- and D2-cells. Functionally, using fluorescent probes for *in vivo* dopamine sensing, we showed that anxiogenic environments trigger higher dopamine release in vHipp and assessed the postsynaptic consequences of these changes in dopamine levels by coupling dopamine imaging with fiber photometry recordings of calcium signals in D1- or D2-expressing vHipp neurons. Bidirectional chemogenetic and optogenetic manipulation of D1- or of D2-neurons' activity causally demonstrated their distinct roles in mediating anxiety and approach/avoidance behaviors. Intriguingly, cocaine exposure modified vHipp dopamine dynamics, with D1- and D2-expressing neurons differently modulating drug-related behaviors, suggesting drug-induced plastic changes in this circuit. Together, we propose that dopamine dynamics in vHipp operate as a feedback loop that bidirectionally tracks anxiety levels to gate exploratory behaviors through differential recruitment of vHipp D1- and D2-neurons, which in turn mediate opposite anxiety-like responses. This work paves the way for further studies of dopamine signal processing in limbic regions, and underscores the complexity of the circuit mechanisms that govern affective states.

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Poster

P705. Anxiety: Pre-Clinical Models

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Program #/Poster #: P705.06

Topic: G.06. Anxiety Disorders

Support: UIC Provost Graduate Research Award
UIC Pathway to an Inclusive Faculty Fellowship
National Institute on Drug Abuse Intramural Research Program Predoctoral Fellowship

Title: Effect of central oxytocin administration on anxiety- and depression-like behavior in C57Bl/6J mice: preliminary evidence for sex-differences

Authors: *K. E. NISBETT[1,2,3], M. TEREUL[3], L. F. VENDRUSCOLO[1], M. E. RAGOZZINO[3], G. F. KOOB[1];
[1]Integrative Neurosci. Res. Br., NIH Natl. Inst. on Drug Abuse IRP, Baltimore, MD; [2]Grad. Program in Neurosci., [3]Dept. of Psychology, Univ. of Illinois at Chicago, Chicago, IL

Abstract: Anxiety and depression are highly prevalent psychiatric disorders worldwide. Evidence indicates that central oxytocin ameliorates social deficits in studies examining social bonding and social anxiety. Although these studies suggest that the therapeutic effects of oxytocin are reliant on social contexts, we hypothesize that the positive effects of oxytocin observed in social contexts are due to intrinsic effects of oxytocin on the neural systems that are related to emotion regulation. The present experiments investigate whether an intracerebroventricular (ICV) infusion of oxytocin (i.e., central action) would reduce anxiety- and/or depression-like behavior in C57Bl/6J mice. Male and female mice received an ICV infusion of vehicle or oxytocin (100, 200 or 500 ng), then they were tested on non-social tests of anxiety and depression, the elevated zero maze (EZM) and the tail suspension test (TST). In males, findings indicate that oxytocin dose-dependently increased the open zone occupancy in the EZM and reduced immobility in the TST, with statistically significant effects at 200 & 500 ng doses. In intact females, oxytocin slightly increased the open zone occupancy in the EZM, whereas it significantly reduced immobility in the TST, having a similar effect at all 3 doses. These initial results demonstrate that central oxytocin is effective in both male and female mice, but these effects vary across sexes and behavioral tests. Further, given the interaction between the oxytocin and estrogen receptor systems, we are exploring whether there is an effect of ovariectomy combined with estrogen replacement therapy on female mice in our mouse model.

Future studies will target specific emotional-related brain regions to help elucidating potential neural mechanisms that underlie the effects of oxytocin on behavior and the observed sex differences.

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Poster

P705. Anxiety: Pre-Clinical Models

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Program #/Poster #: P705.07

Topic: G.06. Anxiety Disorders

Support: P50 AA26117
R37 AA17531
R37 AA17531
R01 AA26551
R21 26455

Title: Sex differences in common rodent assays of anxiety-like behavior following single exposure to predator odor

Authors: *S. R. PITCAIRN[1], J. L. WEINER[2];
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Abstract: Despite the high prevalence of anxiety disorders in women, many preclinical studies focused on anxiety have utilized only male animals in their experiments. The current study sought to characterize potential sex differences in common assays of anxiety-like behavior following a 30-min exposure to either saline or fox urine, a well-validated stressor for rodents. Immediately following exposure to the stressor, male and female Long-Evans rats (n=8/group) underwent behavioral testing using three assays of anxiety-like behavior that were run consecutively, where subjects were first placed in the open field test, followed by the elevated plus-maze, and concluding with the successive alleys test. 90 minutes following completion of the successive alleys test, animals were deeply anesthetized with isoflurane and sacrificed before tissue was collected in order to examine c-fos activity in the basolateral amygdala and ventral hippocampus, two stress-sensitive brain regions known to play a role in anxiety-like behaviors. Results of the behavioral studies demonstrated complex, sexually dimorphic responses. For example, in the open field test there were no effects of odor exposure on anxiety-like behavior (time in center of arena) in either sex but, independent of stressor exposure, females showed a trend of less anxiety-like behavior (p=.06). On the elevated plus-maze, odor-exposed females showed an expected anxiogenic effect while males did not (p=.043). Studies are currently in

progress to quantify c-fos activity as a proxy for neural activation. Although preliminary, the findings from this study suggest that acute exposure to predator odor gives rise to sexually dimorphic behaviors in different assays of anxiety-like behavior, possibly reflecting sex differences in coping strategies employed on these tests.

Disclosures: S.R. Pitcairn: None. **J.L. Weiner:** None.

Poster

P705. Anxiety: Pre-Clinical Models

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Program #/Poster #: P705.08

Topic: G.06. Anxiety Disorders

Support: Canopy Health Innovations

Title: Evidence for a synergistic effect of an edible CBD:THC formulation in reducing anxiety and depressive-like symptoms following chronic stress

Authors: *E. PÉREZ-VALENZUELA, W. RUSHLOW, S. LAVIOLETTE;
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Abstract: Cannabis has shown therapeutic potential in neuropsychiatric pathologies associated with mood and anxiety. The two main phytochemicals in cannabis, cannabidiol (CBD) and delta9-tetrahydrocannabinol (THC), produce distinct effects on a variety of molecular and neuronal pathways associated with anxiety-related disorders. Given the distinct molecular targets of CBD and THC, it has been proposed that combined formulations of THC:CBD have synergistic properties. Nevertheless, evidence for the synergy between CBD and THC in the treatment of mood/anxiety disorders is limited. The aim of this research is to characterize the effects of a 1:100 THC:CBD ratio formulation in anxiety/depressive phenotypes, comparing naïve vs. chronically stressed rats, and to determine the neuronal and molecular mechanisms associated with these effects. Male Sprague-Dawley rats were exposed to unpredictable stress for 14 days. Then, to determine treatment effects on anxiety or depressive-like behaviours, elevated plus-maze test, social interaction task, open field exploration, sucrose preference test, and forced swimming tests were carried out 90 minutes after intake of THC 0.3mg/kg: CBD 30 mg/kg vs. vehicle. After testing, rats were assigned for western blot or electrophysiology experiments. Tissue extractions were taken from prefrontal cortex (PFC), nucleus accumbens (NAc), basolateral amygdala (BLA), and hippocampus. Then, expression and phosphorylation of biomarkers for mood/anxiety disorders including: extracellular-signal-related kinase (ERK1-2), protein kinase B (AKT), and glycogen synthase kinase-3 (GSK-3) were analyzed. For electrophysiological studies, the PFC and ventral tegmental area dopamine neurons were recorded and local field potential (LFP) and single unit activity were analyzed. The oral

formulation of THC:CBD produced an anxiolytic effect in the EPM test and in the social interaction test in stressed-rats. In addition, the edible formulation reversed depressive phenotypes induced by chronic stress. Remarkably, the combination of THC:CBD was superior to either THC or CBD alone, strongly suggesting a synergistic therapeutic potential for this formulation. These changes were also associated with significant expression changes in AKT/GSK-3 signaling pathways mainly in PFC, NAc and hippocampus. Furthermore, the oral formulation of THC:CBD showed differential effects in LFP patterns in the PFC of stressed rats in the Theta and Gamma oscillatory bands. These results show the therapeutic potential of the combination between CBD and THC in pathologies associated with chronic stress such as generalized anxiety disorder or major depression

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Poster

P705. Anxiety: Pre-Clinical Models

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Topic: G.06. Anxiety Disorders

Support: FONDECYT grant N° 1191274

Title: Type-1 corticotropin releasing factor receptor (CRF-R1) expression and neurotransmitter balance in the nucleus accumbens of juvenile rats exposed to social isolation stress

Authors: *F. CALDERON, J. A. ZEGERS, J. A. NOVOA, C. BLANLOT, H. E. YARUR, C. BASTIAS, K. GYSLING;
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Abstract: Rat social isolation is a model of stress commonly used in early stages of life. It has been shown that early and long-lasting intervals of isolation induce anxiety-like behaviors and aberrant neurotransmitter balance in some brain areas during adulthood. Nevertheless, there are a few studies evaluating short periods of isolation, therefore characterization of the latter is scarce and needed. Previous studies of our laboratory have shown that rat social isolation between days 21-32 PND induces an increase in anxiety-like behavior and changes in the balance of neurotransmitter levels. Briefly, isolation induces an increase of dopamine (DA) levels in nucleus accumbens (Nac) in response to a depolarizing potassium stimulus. Interestingly, there are no changes in glutamate (GLU) levels induced by isolation. Furthermore, the effects of a CRF-R1 antagonist upon DA and GLU levels were lost when rats were isolated. The mechanism underlying these changes remains unknown. Preliminary results of our laboratory suggest that these changes rely on the decrease of CRF-R1 expression in Nac. To evaluate this, we used synaptosomes, depleted of their postsynaptic densities, extracted from Nac of isolated and non-

isolated juvenile rats. We performed immunofluorescence in these synaptosomes and measure the expression of CRF-R1 with synapsin I as control. Further studies are needed to strengthen this hypothesis. Funded by FONDECYT grant N° 1191274

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Poster

P705. Anxiety: Pre-Clinical Models

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Topic: G.06. Anxiety Disorders

Support: NIMH MH115409
NIMH MH120637
Brain and Behavior Research Foundation

Title: Ventral tegmental area projection to the basolateral amygdala encodes anxiety-like but not depression-like behaviors

Authors: *M. CAROLE[1], S. E. MONTGOMERY[5], L. LI[2], S. M. KU[5], B. JUAREZ[6], R. DURAND-DE CUTTOLI[2], E. TEICHMAN[2], N. TZAVARAS[3], M. FLANIGAN[5], M. CAI[2], J. J. WALSH[7], S. J. RUSSO[4], E. J. NESTLER[8], E. S. CALIPARI[9], A. K. FRIEDMAN[10], M.-H. HAN[2];
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Abstract: Comorbidity is more the norm than the exception and is strong amongst psychiatric disorders. Anxiety and depression, the highest burden of disability amongst psychiatric disorders, present a high rate of comorbidity, such that 62% of patients with anxiety disorders also experience depressive episodes. This detrimental interplay between anxiety and depression results in the exacerbation of the negative symptoms from one to the other. However, the shared or segregated mechanisms remain largely unknown. Clinical and preclinical studies implicate the dopaminergic system in the emergence of anxiety disorders and depression. In particular, ventral tegmental area (VTA) dopamine neurons which encode rewarding, salient and aversive stimuli, project heavily to emotion-related brain regions, including the medial prefrontal cortex (mPFC), the nucleus accumbens (NAc), and the amygdala to support adaptive behaviors and healthy brain

functions. Here, utilizing the chronic social defeat stress (CSDS) paradigm that induces singular or combined anxiety- and depression-like phenotypes - A group and AD group respectively, we dissect the role of the dopamine subcircuits in the expression of anxiety- or depression-like phenotypes. Utilizing neural circuit-probing techniques, we previously established that the maladaptive firing activity of VTA dopamine neurons projecting to mPFC and projecting to NAc regulates the depressive-like behaviors selectively occurring in AD mice, but not in A mice. In contrast, our current results show that the firing activity of VTA-basolateral amygdala (BLA) neurons is dramatically decreased in both A and AD mice after CSDS. Additionally, using *in vivo* calcium imaging in freely behaving mice, we correlate VTA-BLA circuit activity with the expression of anxiety- but not depressive-related behavioral outcomes. Finally, our optogenetic studies establish bi-directionally the causal link between VTA-BLA subcircuit dysfunction and the anxiety phenotype. Overall, our study identify a novel functional role for VTA-BLA dopamine neurons in controlling anxiety-related behaviors not only in anxiety-alone, but also in anxious-depressive comorbid subjects.

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Poster

P705. Anxiety: Pre-Clinical Models

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Program #/Poster #: P705.11

Topic: G.08. Other Psychiatric Disorders

Support: DOD W81 XWH-10-2-0181

Title: Late positive potential indexes baseline and treatment-related changes in anxiety sensitivity and is modulated by treatment content

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Abstract: Background. Anxiety sensitivity (AS) refers to an individual's tendency to be fearful of anxiety-related bodily sensations, or symptoms that accompany feelings of anxiety (Reiss et al., 1986). These symptoms can be cognitive (i.e., fear of mental dyscontrol), physical (fear of catastrophic physiological outcomes such as heart attack or stroke), or social (concern about negative social judgment and subsequent observable symptoms of anxiety). Cognitive Anxiety

Sensitivity Treatment (CAST) (Schmidt et al., 2014) is a technology-based intervention shown to be efficacious in reducing AS. In recent work by Schmidt (2014), nontreatment seeking participants with elevated AS cognitive concerns participated in one session of CAST and showed significant reductions in AS posttreatment, relative to controls. Current Study. The primary purpose of this study is to assess the late positive potential (LPP) event-related potential (ERP) component, recognized as a marker of motivated attention, as an index of baseline AS, as well as treatment-related change in AS in response to CAST. Methods. In this study 263 participants (with complete EEG data at 1-month follow-up) received either an anxiety reduction intervention of CAST alone (N = 65), a mood intervention of cognitive bias modification interpretation (CBM-I) (N = 66), or a combined CAST and CBM-I interventions (N = 68), as well a repeated contact control group (N= 64). Prior to the intervention, baseline LPP was measured via exposure to affective stimuli (neutral, unpleasant, and pleasant images), and baseline AS recorded using the ASI-3. Results. Across all groups, increased LPP amplitude in response to unpleasant stimuli (UP) was positively correlated with baseline AS ($r=.20$, $p <.001$), but not LPP in response to pleasant or neutral images. At the 1-month follow up, there were different treatment effects based on treatment content: in the CBM-I group, there was an effect in that greater AS reduction at month 1 correlated with early frontal LPP amplitude ($r = -.328$ during our n2 @ Fz, $r = -.387$ during P3 @ Fz, $p < .001$), whereas that effect did not exist frontally in the CAST treatment group ($r = .065$, $p > .05$). The CAST group instead showed AS reduction being related to later parietal LPP effects. Taken together, the Combined group's findings showed a combination of CAST and CBM-I group effects with both early frontal LPP ($r = -.2980$, $p < .05$) and the later LPP effects relating to AS reduction ($r = -.33$, $p < .001$). Discussion. Overall, treatment effects were modulated by treatment content, as CAST yielded one pattern, CBM-I another, and combined CAST + CBM-I displayed an amalgamation of the two in terms of relating AS reduction to EEG amplitude.

Disclosures: D. Butler: None. **A. Pavuluri:** None. **A.A. Vivino:** None. **F. Bautista:** None. **N.B. Schmidt:** None. **E.M. Bernat:** None.

Poster

P731. Neural Mechanisms of Addictive Drugs II

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P731.01

Topic: G.09. Drugs of Abuse and Addiction

Support: Ministerio de Ciencia e Innovación Gobierno de España PID2019-109390RBI00

Title: Structure-activity relationship of novel synthetic cathinones. Selective DAT inhibitors and hybrid compounds at monoamine transporters.

Authors: *N. NADAL-GRATACOS[1,2,3], A. S. SILVA[4], K. JÄNTSCH[4], M. HOLY[4], E. URQUIZU[1,2], M. BUENROSTRO-JÁUREGUI[1], X. BATLLORI[3], X. BERZOSA[3], H. H. SITTE[4], D. PUBILL[2], J. CAMARASA[2], E. ESCUBEDO[2], R. LOPEZ-ARNAU[2];
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Abstract: Several new synthetic cathinones have appeared in the global illicit drug market in the last decades. Synthetic cathinones are designed in order to mimic the effect of already considered illicit compounds such as cocaine, MDMA and methamphetamine. However, the illicit drug market is continually evolving by constantly adding small structural modifications to the common chemical structure of synthetic cathinones. Moreover, and despite the lack of information of their effects, it is widely known that this class of compounds interact with dopamine and serotonin transporters (DAT and SERT) through different mechanisms of action. Thus, the aim of this study was to investigate the *in vitro* structure-activity relationship (SAR) of six novel synthetic cathinones with current popularity as recreational drugs; Pentedrone, Pentylone, N-ethyl-pentedrone (NEPD), N-ethyl-pentylone (NEP), 4-methyl-pentedrone (4-MPD) and 4-methyl-ethylaminopentedrone (4-MeAP), which structurally differ in the absence or presence of different aromatic substituents as well as in the amino terminal group. Human embryonic kidney cells (HEK293) expressing the human isoforms of SERT (hSERT) and DAT (hDAT) were used for the uptake inhibition and release assays. All tested synthetic cathinones presented low IC_{50} values for hDAT and low or negligible activity at hSERT, resulting in high hDAT/hSERT ratio values. At hDAT, the capacity to inhibit [^{3}H]MPP^+ uptake increased with the presence of ethyl amine substitution. When studying [^{3}H]serotonin uptake inhibition at hSERT, ring-substituted cathinones showed higher inhibition potency than their no ring-substituted analogues. Surprisingly, and regarding their releasing properties, besides NEP, all tested compounds evoked serotonin release but negligible effect as DAT substrates. In summary, the present study provides new information about the *in vitro* potency and mechanism of action of some novel synthetic cathinones, which showed “hybrid” properties, acting as DAT blockers but SERT substrates. Moreover, a lack of ring-substitution in the structure of these novel synthetic cathinones together with an increased bulk of the amino terminal group results in higher DAT/SERT ratios, and therefore, higher risk of abuse liability.

Disclosures: N. Nadal-Gratacos: None. **A.S. Silva:** None. **K. Jäntsch:** None. **M. Holy:** None. **E. Urquizu:** None. **M. Buenrostro-Jáuregui:** None. **X. Batllori:** None. **X. Berzosa:** None. **H.H. Sitte:** None. **D. Pubill:** None. **J. Camarasa:** None. **E. Escubedo:** None. **R. Lopez-Arnau:** None.

Poster

P731. Neural Mechanisms of Addictive Drugs II

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Program #/Poster #: P731.02

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH R01 MH64489
NIH R21 DA046044
NIH T32 DA031115

Title: Role of organic cation transporter 3 in ethanol and cocaine co-abuse

Authors: *N. CLAUSS[1], A. OWENS[1], M. VITELA[1], M. A. BOWMAN[1], G. GOULD[1], W. KOEK[2], L. C. DAWS[1];
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Abstract: Concurrent use of cocaine and alcohol is a major cause for emergency hospitalization, underscoring a vital need to understand the mechanistic basis of this highly addictive, and dangerous drug combination. Both cocaine (blocker of dopamine, norepinephrine and serotonin transporters, DAT, NET and SERT, respectively) and ethanol (EtOH) increase extracellular dopamine (DA), norepinephrine and serotonin. However, we and others, find no evidence for EtOH interacting with DAT, NET or SERT, suggesting that EtOH may be acting elsewhere to inhibit uptake of these monoamines. Organic cation transporter 3 (OCT3) is emerging as an important player in regulation of monoamine signaling. However, whether OCT3 contributes to actions of abused drugs that act primarily by increasing extracellular levels of monoamines, remains unclear. To establish proof-of-principle for this idea, we conducted studies to determine the ability of EtOH to inhibit uptake of the prototypical cation [^{3}H]1-methyl-4-phenylpyridinium ([^{3}H]MPP$^+$) into cultured HEK293 cells stably overexpressing human OCT3, and found the IC_{50} to be 4 mM, supporting a role for OCT3-dependent actions of EtOH, and showing that concentrations of EtOH reaching brain following behaviorally relevant doses may be sufficient to engage OCT3. We also found that cocaine does not have activity at OCT3 (Mayer et al., 2018, PMID: 29773909) raising the possibility that EtOH may interact with OCT3 to inhibit uptake of monoamines, potentially increasing the addictive properties of cocaine and propagating the concurrent use of these drugs. To this end we used high-speed chronoamperometry to interrogate the effects of local application of EtOH, cocaine, and their combination on clearance of exogenously applied DA from extracellular fluid in striatum of male and female constitutive OCT3+/+ and OCT3-/- mice *in vivo*. We found that in both male and female OCT3+/+ mice, EtOH inhibited DA clearance and enhanced the ability of cocaine to inhibit DA clearance, effects that were lost in OCT3-/- mice. Behavioral data in male and female OCT3+/+ mice reveal that the combination of EtOH (1000 mg/kg) and cocaine (3.2 mg/kg), doses which by themselves did not induce conditioned place preference (CPP), produced robust CPP. CPP studies in OCT3-/- mice are ongoing, but so far demonstrate no development of CPP, regardless of treatment. Taken together, results suggest that potentiation of the neurochemical and behavioral effects of cocaine by EtOH are OCT3 dependent. OCT3 may be a putative target for therapeutic intervention in the treatment of EtOH and cocaine co-abuse.

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Poster

P731. Neural Mechanisms of Addictive Drugs II

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Program #/Poster #: P731.03

Topic: G.09. Drugs of Abuse and Addiction

Support: Tactogen
NIH grant DA 00522-14

Title: Benzofuran derivatives are potent serotonin releasers and substitute for the discriminative stimulus effects of MDMA in rats.

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Abstract: 3, 4-Methylenedioxymethamphetamine (MDMA) is currently under evaluation in phase III clinical trials for the treatment of post-traumatic stress disorder (PTSD) and is expected to be approved by the FDA for clinical use in the near future. MDMA is also a popular abused substance with risks for cardiovascular toxicity and neurotoxicity, particularly when misused at higher doses. Characterization of the behavioral and neurochemical effects of novel psychoactive substances is an essential step in the development of safer alternative therapeutic agents. Drug discrimination is a preclinical behavioral assay with pharmacological specificity for characterizing *in vivo* drug actions in the central nervous system. This project implemented rodent drug discrimination studies and serotonin transporter assays to characterize the enantiomers of 5-(2-methylaminopropyl) benzofuran (5-MAPB) and 5-(2-methylaminobutyl)benzofuran (5-MBPB), two benzofuran derivatives with potential MDMA-like effects. Eight male Sprague-Dawley rats were trained in a standard two-lever operant drug discrimination procedure under a fixed ratio 20 schedule of food reinforcement to discriminate MDMA (1.5 mg/kg) from saline. Once criteria for stimulus control were established, stimulus substitution tests were conducted with (RS) 5-MAPB, (R)-5-MAPB, (S)-5-MAPB, (R)-5-MBPB, and (S)-5-MBPB. All substances produced dose-dependent increases in MDMA-lever responding and full substitution at the highest dose assessed with minimal effects on response rate. The 5-MAPB and 5-MBPB enantiomers differed slightly in potency. Serotonin release assays using rat synaptosomes and [3H]serotonin indicated all substances are substrate-type releasers of serotonin with nanomolar potency. S-enantiomers appeared to have a somewhat higher potency than their corresponding R-enantiomers. The benzofuran scaffold may allow the development of substances that retain MDMA-like therapeutic effects while reducing toxicities associated with MDMA.

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Poster

P731. Neural Mechanisms of Addictive Drugs II

Location: Virtual Only

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Program #/Poster #: P731.04

Topic: G.09. Drugs of Abuse and Addiction

Support: Alcohol Research Council of the Swedish Alcohol Retailing Monopoly
European Foundation for Alcohol Research (EA 11 30)
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Swedish Brain Foundation
Research Foundations of Bertil Hållsten
Swedish Research Council (VR: 2015-00495)

Title: Gene expression of Vesicular Glutamate Transporter 2 in the rat ventral tegmental area after episodic adolescent exposure to nicotine and alcohol

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Abstract: Adolescence is a highly sensitive period, during which initiation of use/co-use of alcohol and nicotine commonly occurs in an episodic manner and can imprint vulnerability to the developing brain. Alterations in glutamatergic neurotransmission have been implicated in various phases of addiction cycle, yet little is known about the interactive effect of both nicotine

and alcohol on it. A marker of the glutamatergic phenotype is the Vesicular Glutamate Transporter 2 (VGLUT2) which is expressed in the ventral tegmental area (VTA), in glutamatergic-only but also in dopaminergic neurons. Differential VTA *VGLUT2* expression has been observed after chronic nicotine use and co-abuse with alcohol in human post-mortem brains. The present study aimed to investigate whether *Vglut2* gene expression in the VTA of adolescent outbred male rats is affected by prolonged episodic nicotine and/or alcohol administration. A total of forty animals were used, randomly assigned to four experimental groups (n = 10, per group) exposed to either: i) alcohol-only; ii) nicotine-only; iii) combination of alcohol and nicotine; or iv) control (water and saline) for three consecutive days per week for six weeks during adolescence. *Vglut2* mRNA was assessed in the anterior and the posterior VTA by use of double fluorescent or combined fluorescent/chromogenic *in situ* hybridization followed by automated cell counting. Differences on *Vglut2*-positive cells (%) among groups were assessed using one-way ANOVA, followed by Tukey *post-hoc* tests. Between-group comparisons on glutamatergic and dopaminergic neuronal subpopulations were performed using the independent student t-test. The profile of glutamatergic and dopaminergic neurons varied among the sub-nuclei of the VTA. *Vglut2* expression in the glutamatergic neurons was borderline upregulated in the lateral anterior, but not the posterior, VTA of rats exposed to both nicotine and alcohol compared to the control group. Contrariwise, alcohol alone and the combination of drugs borderline affected the glutamate-releasing dopaminergic neurons towards the opposite direction. The present preliminary results support the VTA heterogeneity, with different effects of alcohol and nicotine in the anterior compared to the posterior VTA. Furthermore, they hint towards an early effect of prolonged episodic alcohol and nicotine adolescent exposure on the distinct VTA glutamatergic/dopaminergic subpopulations. Determining genetic signatures of key-neuronal markers such as *Vglut2*, in key reward brain regions can contribute to further understanding of the biological underpinnings of drug addiction.

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Poster

P731. Neural Mechanisms of Addictive Drugs II

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA046794
NIH Grant HD070888

Title: Fluoxetine potentiates gene regulation by chronic oral methylphenidate treatment in the rat striatum

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Abstract: The psychostimulant methylphenidate (MP, Ritalin) is used in the treatment of attention-deficit hyperactivity disorder (ADHD) and as a cognitive enhancer in the healthy. MP, like cocaine, acts by blocking the reuptake of dopamine. However, unlike cocaine, MP does not affect serotonin. Serotonin contributes to addiction-related gene regulation by cocaine. Thus, the lack of a serotonin effect may explain MP's more moderate impact on gene regulation and addiction liability. Previously, we showed that enhancing serotonin action by adding a selective serotonin reuptake inhibitor (SSRI), fluoxetine (FLX), to MP indeed potentiates MP-induced gene regulation in the striatum in rats. However, these studies used intraperitoneal (IP) drug administration, which is more relevant for MP abuse. IP administration produces drug spikes that are known to have a greater impact on gene regulation. In clinical treatments, however, MP and FLX are typically taken orally, which produces a slower bioavailability of the drugs and avoids spikes. In the present study, we investigated whether chronic oral administration of MP and FLX altered gene regulation in the striatum. MP (30/60 mg/kg/day), FLX (20 mg/kg/day) and MP+FLX were administered in rats' drinking water for 8h/day over 4 weeks. We assessed the expression of the neuropeptides dynorphin and substance P (both markers for striatal direct pathway neurons) and enkephalin (indirect pathway) by in situ hybridization histochemistry. Chronic oral MP alone produced a tendency for increased dynorphin and substance P expression and no changes in enkephalin expression. Oral FLX alone did not increase gene expression. In contrast, when given together, FLX greatly enhanced oral MP-induced expression of dynorphin and substance P and to a lesser degree enkephalin. Thus, FLX potentiated oral MP-induced gene regulation predominantly in direct pathway neurons, mimicking cocaine effects. The three functional domains of the striatum were differentially affected: While present throughout the striatum, MP+FLX-induced increases in dynorphin and substance P expression were maximal in sensorimotor sectors and minimal in limbic sectors. In contrast, increases in enkephalin expression occurred in associative sectors. MP+SSRI concomitant therapies are indicated in ADHD/depression comorbidity and other disorders, and co-exposure also occurs with MP misuse as a cognitive enhancer by patients on SSRIs. Our findings indicate that chronic MP+SSRI combinations, even given orally, may enhance addiction-related gene regulation.

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Poster

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CAPES/CNPQ

Title: Sex, but not chronic cocaine administration, alters allopregnanolone levels in rat brain tissue and serum

Authors: *M. H. MCFARLAND[1], M. M. MACHADO[3], C. C. CARR[1], G. M. SANSBURY[1], K. C. MUSSELMAN[1], G. BOERO[4], T. K. O'BUCKLEY[1], A. MORROW[5], D. L. ROBINSON[2]; [2]Bowles Ctr. for Alcohol Studies, [1]Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; [3]Univ. Federal De Sao Paulo, Diadema, Brazil; [4]Dept. of Psychiatry, Univ. of North Carolina At Chapel Hill, Chapel Hill, NC; [5]Psych, Pharmacol & Ctr. Alcohol, UNC Sch. of Med., Chapel Hill, NC

Abstract: Neurosteroids are compounds synthesized within the brain that influence neuronal activity, in part, via actions at γ-aminobutyric acid (GABA) type A receptors. Multiple psychiatric disorders are associated with altered brain and serum levels of neurosteroids. Furthermore, the endogenous GABAergic steroid (3α,5α)-3-hydroxy-5-pregnan-20-one, or allopregnanolone, a metabolite of progesterone, has emerged as a clinically beneficial therapeutic for the treatment of psychiatric disorders, such as post-partum depression and substance use disorders. Although allopregnanolone can reduce cocaine-seeking and reinstatement in rats, the mechanism by which it exerts its effects remains unclear. Clinically, chronic cocaine use was correlated with decreased levels of pregnenolone, a precursor to allopregnanolone, and increasing allopregnanolone levels via progesterone administration in humans with a history of cocaine use resulted in reduced cocaine craving and stress arousal. The present study tested the hypothesis that chronic cocaine exposure in male and female rats would reduce levels of allopregnanolone in serum and brain regions involved in dopaminergic transmission and neurosteroidogenesis, such as olfactory tubercle, cortex, dorsal and ventral striatum, and midbrain. To test this hypothesis, we administered 15 mg/kg cocaine or saline to Sprague-Dawley female and male rats daily for 14 days consecutively. We recorded locomotor activity and stereotyped behavior each day to assess for cocaine sensitization. We observed typical behavioral neuroadaptations consistent with cocaine sensitization, including increased locomotion and stereotyped behavior in cocaine-exposed animals over the 14 days of administration. After allopregnanolone extraction from brain tissue and serum, we observed no significant differences in allopregnanolone levels between cocaine and saline groups in serum or any brain regions analyzed. We did, however, identify a main effect of sex in serum, olfactory tubercle, and cortex, but not in midbrain or ventral and dorsal striatum. These findings revealed that allopregnanolone levels vary across brain regions and by sex, which may play a part in sex differential responses to stress.

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Poster

P731. Neural Mechanisms of Addictive Drugs II

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CIC-UMSNH 30.2

Title: Participation of purinergic $P2X_{2/3}$and $P2X_3$receptors in toluene-induced acute and chronic nociception in rats

Authors: *M. Y. GAUTHEREAU-TORRES[1], Y. S. RODRIGUEZ-HERREJON[1], V. GRANADOS-SOTO[4], D. GODINEZ-HERNANDEZ[2], C. J. GUTIERREZ-GARCIA[5], L. F. ORTEGA-VARELA[3];
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Abstract: Toluene is the most misused solvent. It can be found as part of multiple products such as thinner, adhesives, gasoline, and cleaning products. The abuse of this solvent is considered a public health problem, mainly between children and adolescents. Toluene is known to affect the function of the pronociceptive purinergic $P2X_{2/3}$ and $P2X_3$ receptors by activating or inhibiting them, respectively. There is evidence indicating that exposure to toluene can cause an increase in nociception through neurotransmitter systems such as serotonergic system. However, it is unknown whether other neurotransmitter systems are involved in the pronociceptive effect of toluene. The purpose of this study was to assess the participation of the local peripheral purinergic $P2X_{2/3}$ and $P2X_3$ receptors in the effect of toluene exposure on nociception, hyperalgesia and allodynia. Female Wistar rats were acutely exposed to toluene (6000 ppm) or air and some groups were treated with a $P2X_{2/3}$ and $P2X_3$ receptor antagonist (A-317491). Acute nociception was assessed using the formalin test and subsequently hyperalgesia and allodynia were evaluated with von Frey filaments in the same test. Acute solvent exposure increased

formalin-induced acute and chronic nociception in rats. Toluene pronociceptive acute and chronic effects were blocked in a dose-dependent fashion with A-317491. Data suggest that peripheral $P2X_{2/3}$ and $P2X_3$ receptors participate in the development of toluene-induced pronociceptive effects in rats.

Disclosures: M.Y. Gauthereau-Torres: None. **Y.S. Rodriguez-Herrejon:** None. **V. Granados-Soto:** None. **D. Godinez-Hernandez:** None. **C.J. Gutierrez-Garcia:** None. **L.F. Ortega-Varela:** None.

Poster

P927. Optical Methodologies

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Topic: I.04. Physiological Methods

Support: FET OPEN Grant 828972
ERC Grant 677683
NIH Grant 1UF1NS108177-01
NIH Grant U01NS094190

Title: Collecting Raman signals from deep brain regions

Authors: *F. PISANO[1], M. S. ANDRIANI[1], M. AL-MASMUDI MARTÍN[2], T. JURADO-PARRAS[3], A. BALENA[1], M. PISANELLO[1], M. F. KASHIF[4], A. SANCHEZ-AGUILERA[3], E. R. SEBASTIAN[3], A. D'ORAZIO[4], F. TANTUSSI[1], M. GRANDE[4], F. DE ANGELIS[1], M. VALIENTE[2], L. DE LE PRIDA[3], M. DE VITTORIO[1], F. PISANELLO[1];
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Abstract: Raman spectroscopy is a powerful, *label free* technique to probe the molecular composition of a specimen. Recent studies have shown the potential of Raman spectroscopy to investigate the onset of neuropathological conditions emerging from brain tumors or traumatic brain injury. To exploit the full potential of this approach, it is crucial to access deep brain regions that lay beyond the reach of conventional Raman micro-spectroscopy. However, even the most advanced Raman probes, which are designed to provide intraoperative surgical guidance in humans, are bulky and expensive and are unsuited to retrieve Raman signals from deep brain regions in small animals, e.g. the mouse. To circumvent these limitations, we propose the use of a minimally invasive, disposable tapered optical fiber as an implantable probe to collect volumetric Raman signal at depth. We show that our approach not only can resolve the cytoarchitectonic layering of the mouse cerebral cortex, but also provides novel information on biochemical alterations linked with traumatic brain injuries and with the formation of brain

metastasis from different primary tumor (e.g. melanoma or lung). We believe our work is a significant contribution towards the future establishment of novel strategies for multimodal evaluation of the brain with a broad array of applications compatible with the study of the physiological state as well as the impact of any disorder affecting this organ.

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Poster

P927. Optical Methodologies

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Topic: I.04. Physiological Methods

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Title: Wearable high-density diffuse optical tomography (WHD-DOT) instrument for mapping human brain activity

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Abstract: In recent years, functional neuroimaging has gone beyond the field of cognitive neuroscience and is increasingly being used in clinical applications for diagnosis and treatment planning. Its expanding application outside traditional research lab settings requires instruments with the flexibility to accommodate various clinical populations and allow a wide range of behavioral paradigms in naturalistic environments. The logistics of traditional neuroimaging technologies (such as fMRI, PET and MEG) exclude participants with metal implants or movement disorders and those who are unable to lie still in the scanner. Optical neuroimaging provides alternative methods for human brain mapping where gold-standard imaging modalities are contraindicated. Functional near-infrared spectroscopy (fNIRS) is one such method that is safe, lightweight, and portable. However, most fNIRS systems have low channel counts that limit resolution, field of view, and depth sensitivity. High-density diffuse optical tomography (HD-DOT), a form of fNIRS with high spatial density and increased channel count, provides

improved image quality and brain specificity but uses bulky optical fibers, which restrict subject movement and portability. We developed a wearable HD-DOT (WHD-DOT) instrument that combines the portability of fNIRS and the image quality of HD-DOT. Our design replaces fibers and rackmount hardware with modules that integrate the electronics for optical sources and detectors into tiny packages that fit on the head. Each module works like a smart comb with cylindrical tips that brush through hair and maintain stable optode-scalp coupling for optimal light detection. Multiple modules are combined using a custom FPGA board, which simultaneously controls sources and decodes signals from detectors. Real-time data from the WHD array is transmitted to a laptop via WiFi. Compact rechargeable batteries, stored in a small backpack, powers the whole system. We created a WHD-DOT instrument with 32 sources and 32 detectors at 13 mm optode spacing. The modular structure of the WHD patches facilitates scaling to a whole-head field of view with up to 144 sources and 144 detectors. In-vivo measurements were conducted to characterize system performance, resulting in dynamic range, cross talk and effective noise levels similar to fiber-based HD-DOT. To establish imaging feasibility, we validated mapping performance using retinotopic mapping of the visual cortex.

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Poster

P927. Optical Methodologies

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Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P927.03

Topic: I.04. Physiological Methods

Support: NIH Grant R01NS090874 NINDS

Title: Imaging Cerebral Blood Flow Using Speckle Contrast Optical Tomography: Simulation in a Human Head Model

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Abstract: The development of real-time measures of cerebral blood flow (CBF) in clinics is desired since CBF is a crucial biomarker for brain diseases. Speckle contrast optical tomography

(SCOT) is a non-invasive imaging modality for mapping CBF via analysis of dynamic speckles; moreover, it has the potential for imaging at the bedside and has cost advantages and improved signal-to-noise ratio (SNR) compared to diffuse correlation spectroscopy (DCS), a popular optical method for imaging CBF. It also solves the limitations of positron emission tomography, the gold standard of imaging CBF, by enabling continuous measurements without ionizing radiation. High-density SCOT (HD-SCOT) has been studied in rodents, but the current free-space design of SCOT is not ideal for large-area brain imaging in humans. A fiber-based HD-SCOT is considered a potential solution where the fibers can comb through the hair and the fiber cap can be conformed to the complex surface of the human head. However, anatomical based reconstructions for HD-SCOT system on a human head have yet to be fully developed. In this study, we developed a pipeline for simulating HD-SCOT measurements and reconstructed images, and then we used the simulations to investigate the CBF imaging performance. Our pipeline is based on NIRFASTer and NeuroDOT modeling packages, and we simulated imaging with an array of 24 sources by 28 detectors and an anatomical head model. We studied the imaging depth sensitivity under varied source-detector distances and system exposure times with a simulated perturbation of CBF and demonstrated that the superficial cortical region of the brain can be measured with second nearest neighbors (source-detector distance = 29 mm). Simulated images showed spatial resolution of approximately 12 mm full-width-half-maximum at the depth of 10 mm, and reduced localization error when including larger source-detector distances. However, noise in an imaging optical system can prohibit the goal to use larger source-detector distances because of the attenuated signal-to-noise ratio (SNR). The system exposure time plays an important role in SNR since both the measurement amplitude and noise of an HD-SCOT system are strongly dependent on the exposure time. We evaluated SNR vs exposure time and found that the measurement SNR is approximately 1000x greater for an exposure time of 1ms relative to 1 μs. These results can be used to guide future instrument designs, including the optimal exposure time and available source-detector distances, and it is a powerful tool for studying the performance of HD-SCOT since different noise models can be easily incorporated into the pipeline.

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Poster

P927. Optical Methodologies

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P927.04

Topic: I.04. Physiological Methods

Title: Measuring simultaneous neural activity and blood flow in awake freely behaving animals

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Abstract: Neurovascular coupling, the process of localized change in blood flow following neuronal activity, ensures active regions of the brain are supplied with abundant levels of oxygen, glucose and substrates in a timely manner. Pathological conditions such as stroke, tumor, hypertension, Vascular Cognitive Impairment and Dementia (VCID) and Alzheimer's disease disrupt this intricate process impacting brain function. The complex nature of how this disruption occurs, however, remains elusive. Studying neurovascular coupling requires precise measurements of the spatial and temporal relationships of neuronal activity and changes in blood flow. We have developed a dual color miniscope, the nVueTM system, that allows simultaneous imaging of the neural activity and blood flow in animals during free behavior, enabling the investigation of neurovascular coupling. The nVue system acquires dual color data by multiplexing two LEDs at a high frame rate enabling direct visualization of both blood flow and calcium activity. We have also developed analysis algorithms for accurate and precise measurement of changes in vessel diameter and red blood cell (RBC) velocity. To validate that we could detect changes in blood flow alongside changes in neural activity, we injected a blood plasma labeling dye, Rhodamine-dextran, in GCaMP expressing mice via the tail vein. We then simultaneously imaged GCaMP and Rhodamine-dextran signals using the nVue system in freely behaving animals in baseline and amphetamine conditions. We observed 1) changes in blood flow correlated with increased neuronal firing and 2) vasodilation and increased blood flow following amphetamine administration compared to saline controls. This new application of the nVue miniscope system opens up new ways to study functional circuit data with vascular dynamics in cortical and deep brain regions in awake animal paradigms for both basic and translational neuroscience areas.

Disclosures: S. Gulati: A. Employment/Salary (full or part-time):; Inscopix. **O. Miller:** A. Employment/Salary (full or part-time):; Inscopix. **D. Cheng:** A. Employment/Salary (full or part-time):; Inscopix. **S. Malek:** A. Employment/Salary (full or part-time):; Inscopix. **S. Noguchi:** A. Employment/Salary (full or part-time):; Inscopix. **N. Adil:** A. Employment/Salary (full or part-time):; Inscopix. **B. Boivin:** A. Employment/Salary (full or part-time):; Inscopix. **S. Neufeld:** A. Employment/Salary (full or part-time):; Inscopix. **A.M. Stamatakis:** A. Employment/Salary (full or part-time):; Inscopix.

Poster

P927. Optical Methodologies

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Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P927.05

Topic: I.04. Physiological Methods

Support: NSERC
Quebec Bio-Imaging Network (QBIN)

Title: High-throughput automated cages for longitudinal monitoring of neurovascular coupling in mice

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Abstract: In neuroscience, wide-field optical imaging in awake mice is an efficient technique to monitor the neural dynamic in the neocortex. It is usually performed on head-fixed awake mice implanted with a titanium bar and a chronic cranial window giving access to the brain. Mice are manually fixed to the apparatus via the bar to perform various tasks related to observing or manipulating brain activity through optogenetics. These protocols are very time-consuming for the experimenter and cause a lot of variabilities. A new strategy that emerged recently was to design a device able to automate this process, where mice set themselves to perform tasks while allowing massive data acquisition (Murphy et al. 2020 eLife). Automation also helps to increase the reproducibility of the experiments. Our goal was to optimize this technology and combine it with visual discrimination tasks and visual stimulation paradigms in the context of a longitudinal study exploring stroke. We created an automatic cage composed of two compartments, a weighing tunnel and a fixation tunnel having the only source of water, the wide-field optical imaging system, and a LCD screen. RFID readers are located on the two tunnels to identify the mice. When the mice enter into the fixation tunnel, the fixation system locks the head and starts the imaging and visual tasks, while the water is given. Besides the technical development of the device, the main challenge lies in the learning procedure of this task by the mice. Seven mice were implanted and then placed in a training cage where they were accustomed to drinking at the end of a passive tunnel. Then the mice were placed in the real automatic cage where the fixation sound was activated on the fourth day. After getting used to it and successful drinking, partial fixation was introduced, then hard fixation to stabilize the heads of mice for mesoscopic imaging. Widefield intrinsic signals imaging was introduced while the mice were head-fixed during 45s, using red, green and orange light focused on the dorsal cortex. Within the automatic cage, 6 mice out of 7 performed voluntary head fixations at an average rate of 27 fixations per day and per mouse. Considering the fixation time of 45s, it represents around 20min/day/mouse. Furthermore, over 5000 fixations with intrinsic signal imaging were registered. This system has great potential for longitudinal optical imaging studies in awake mice. It can then improve the research on the evolution of neurological disorders such as stroke in the visual cortex, their effect on cognition and the search for new treatments. The large amount of data generated can easily be shared and thus accelerate discoveries in the field of vision neurosciences.

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Poster

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Program #/Poster #: P927.06

Topic: I.04. Physiological Methods

Support: Natural Sciences and Engineering Research Council of Canada
Fonds de recherche du Québec - Nature et technologies

Title: Simple movement correction method for laser speckle contrast imaging of cerebral blood flow in cranial windows in rodents

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Abstract: Laser speckle contrast imaging (LSCI) is a widely used method to monitor blood flow in biological tissue. One of its main research applications is the mapping of cerebral activity by the measurement of changes in cerebral blood flow (CBF), which serve as a proxy for neuronal activation through neurovascular coupling. A major drawback of LSCI is its susceptibility to movement artifacts, a problem that is seldom considered despite the technique's large adoption. Movement artifacts are especially prevalent in stimulus response experiments, in which the hemodynamic response to a sensory stimulus is to be measured. In such studies, movement makes it hard to use LSCI to study the temporal and spatial dynamics of the CBF response, which are crucial to understand how functional imaging signals relate to neuronal activity. Here, we demonstrate a simple motion correction method that allows to describe both spatially and temporally the onset of the CBF response using LSCI, which is usually obscured by motion artifacts. The method is based on identifying a scattering region inside the imaged field of view in which the speckle contrast varies independently of CBF and then regressing out the mean-subtracted signal from this region. In a mouse cranial window preparation in which the edges of the window are fixed with dental cement, a common preparation in awake animal studies, we show that the average signal outside the exposed brain correlates strongly with head movement and hence forms an appropriate movement regressor. We demonstrate that the regression method corrects stimulus-locked motion artifacts, increases the signal-to-noise ratio, and makes CBF responses more physiologically plausible, revealing the hemodynamic lag that was masked by movement. We further validate the method by simultaneously measuring relative changes in cerebral blood volume (CBV) using intrinsic optical imaging and show that correcting the speckle contrast response increases its concordance with the CBV response, consistent with the fact that the two are physiologically linked. Finally, we show that the regression method performs better than alternative methods including image realignment and global signal regression. Our work highlights the importance of having a proper movement correction strategy when performing LSCI in awake animals and identifies the regression of flow-independent speckle contrast variations as a simple and accessible way to improve the quality of LSCI data.

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Poster

P927. Optical Methodologies

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Program #/Poster #: P927.07

Topic: I.08. Methods to Modulate Neural Activity

Support: SPARC Program OT2OD024912
NIH Grant R01NS097184
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Einstein-Sinai Diabetes Research Center Grant P-30 DK020541

Title: Blood glucose regulation by magnetogenetic stimulation of pancreatic nerves

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Abstract: There is substantial evidence that neural regulation plays a critical role in glucose metabolism and hormone release. Targeting neural populations innervating the pancreas could provide therapies for diabetes and other metabolic conditions. However, our understanding of the precise physiological roles of pancreatic nerves is incomplete because we lack tools to study these populations in a non-invasive, targeted, temporally controlled manner. Here, we validated and applied a novel magnetogenetic neuromodulatory construct *in vitro* and *in vivo* to discern the roles of defined pancreatic nerves in glucose metabolism.
The construct, coding for the TRPV1 ion channel fused to a nanobody binding endogenous ferritin (NbTRPV1), was transfected into Neuro2A cells and primary mouse DRG neurons. Magnetic stimulation significantly increased intracellular calcium in cells expressing NbTRPV1. For *in vivo* delivery, cre-dependent NbTRPV1 was packaged into AAV8; *ex vivo* calcium imaging of celiac ganglia confirmed magnet activation of pancreas-projecting neurons expressing NbTRPV1.
In vivo, magnet treatment of mice expressing NbTRPV1 in β cells increased plasma insulin and improved glucose tolerance in males and females, validating the efficacy of this construct to regulate cell activity in the pancreas of freely moving mice.
When NbTRPV1 was delivered to all pancreas-projecting efferent neurons, glucose clearance was significantly improved, mediated by suppression of glucagon secretion. Next, we targeted expression to pancreatic parasympathetic efferent innervation; magnet treatment of mice expressing NbTRPV1 in parasympathetic efferent pancreatic neurons significantly improved

glucose tolerance via increased insulin secretion, without impact on plasma glucagon. Finally, NbTRPV1 was targeted to pancreatic vagal sensory neurons in either the left or right nodose ganglion. With magnet treatment, these mice showed impaired glucose tolerance, reduced insulin secretion and increased glucagon secretion in the face of glucose challenge. These data demonstrate for the first time a method for remote, temporally controlled, targeted modulation of pancreatic islets and nerves to regulate blood glucose. These are also the first studies to begin parsing out the physiological roles of vagal sensory neurons projecting *specifically* to the pancreas, without off-target effects resulting from stimulation or ablation of the entire vagus nerve. This approach can be used to study the contribution of pancreatic nerves in a wide range of diseases, from diabetes to pancreatic cancer, as well as other peripheral nerves and conditions relevant to their pathophysiology.

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Poster

P966. Computational and Analysis Tools V

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P966.01

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

Support: NSF Grant EEC-1801666
NSF Grant DBI- 1659831
NSF Grant OAC-1730655
NIH Grant MH122023
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Title: Neural Engineering in K-12

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Abstract: STEM engagement in U.S. K-12 classrooms has increased over the past decade thanks to efforts from the National Science Foundation and the National Institute of Health and the adoption of Next Generation Science Standards (NGSS) into the classroom. While NGSS has embedded engineering, neuroscience, and computer science skills and topics into traditional K-12 course requirements, the integration of these skills and topics by schools and teachers is not

standardized. Teachers and schools are experiencing an increasing pressure to incorporate these STEM concepts in a way that students can understand and enjoy but lack both resources and adequate STEM expertise. While many programs, lessons, and activities used to embed STEM into K-12 exist, many of these resources are unavailable to low-income schools due to factors including cost and classroom space requirements. We introduce an open-source eBook complete with freely accessible STEM lessons that introduce students to concepts that are relevant to the new K-12 standards related to engineering and neuroscience. These lessons are aligned with NGSS, Common Core, and state standards, and each lesson includes a set of lecture slides, assessments, and an engaging, hands-on activity.

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Poster

P966. Computational and Analysis Tools V

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Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P966.02

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

Support: Beckman Young Investigator Award

Title: Fiola: an accelerated pipeline for fluorescence imaging online analysis

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Abstract: Optical microscopy methods such as calcium and voltage imaging already enable fast activity readout (30-1000Hz) of large neuronal populations using light. However, the lack of corresponding advances in online algorithms has slowed progress in retrieving information about neural activity during or shortly after an experiment. This technological gap not only prevents the execution of novel real-time closed-loop experiments, but also hampers fast experiment-analysis-theory turnover for high-throughput imaging modalities. The fundamental challenge is to reliably extract neural activity from fluorescence imaging frames at speeds compatible with new indicator dynamics and imaging modalities. To meet these challenges and requirements, we propose a framework for Fluorescence Imaging OnLine Analysis (FIOLA). FIOLA exploits computational graphs and accelerated hardware to preprocess fluorescence imaging movies to extract neural activity at speeds in excess of 300Hz on standard fields of view for calcium imaging and at over 400Hz for voltage imaging movies. We evaluate FIOLA on ground truth

data and real datasets, demonstrating reliable and scalable performance. Our methods provide the computational substrate required to interface precisely large neuronal populations and machines in real-time, enabling new applications in neuroprosthetics, brain-machine interfaces, and experimental neuroscience. Moreover, this new set of tools is poised to dramatically shorten the experiment-data-theory cycle by providing immediate feedback on the activity of large neuronal populations at experimental time.

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Poster

P966. Computational and Analysis Tools V

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Program #/Poster #: P966.03

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

Support: NIH/NINDS R01 NS109552
NSF HRD-1547723

Title: Parameter estimation and uncertainty quantification of Hodgkin-Huxley style neuron models using the Markov chain Monte Carlo method

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Abstract: Experimental data based parameter search for Hodgkin-Huxley style neuron (HHN) models is a major challenge for neuroscientists. Current search strategies are often computationally expensive, slow to converge, poorly handle nonlinearities in the objective function or require good initial guesses. Most importantly, none of the existing approaches offer quantification of uncertainties in parameter estimates even though such uncertainties are of immense biological significance. We propose a novel state-of-the-art method for uncertainty quantification in a Bayesian framework using the Markov chain Monte Carlo (MCMC) approach. This approach incorporates prior knowledge about model parameters (as probability distributions) and aims to map the prior to a posterior distribution of parameters informed by both the model and the data. Furthermore, using the adaptive parallel tempering strategy for MCMC, we tackle the highly non-linear and noisy HHN cost-function. We tested the robustness of our approach using the voltage trace data generated from a well studied 9-parameter HHN model using four levels of injected currents (0.0, 0.1, 0.2 and 0.3 nA). Each test consisted of

running the ground truth with their respective currents to estimate the model parameters. Each training run used 24 to 32 chains and generating 10000 to 14000 samples.We found that the MCMC algorithm was able to converge to an optimal parameter set with a loss value of 0.0008, 0.0002, 0.0348, 0.0013 for each of the four injected currents respectively. Our search strategy also produced other solutions with acceptable losses (e.g., for 0.0 nA, 889 solutions were within 0.5% of the best solution and 1595 solutions with 1% of the best solution). Thus, in addition to producing a single optimal solution, the MCMC search provided a "landscape" of the possible parameters sets with acceptable loss values in a tractable manner. Our approach, for the first time, is able to obtain an intelligently sampled global view of the solution distributions within a search range in a single swoop. Furthermore, we also demonstrate a practical strategy to unravel hitherto 'unseen' solution spaces which can serve to better inform and guide future experiments.

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Poster

P966. Computational and Analysis Tools V

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Program #/Poster #: P966.04

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

Support: NIH Grant NS116883

Title: Convergent mapping dominates cerebro cerebellar connectivity

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Abstract: The cerebro-ponto-cerebellar system entails aseries of closed loops, creating anetwork inwhich cerebral and cerebellar regions are organized inaone-to-one mapping (Schmahmann&Pandya, 1995;Buckner et al. 2011). However, other lines of evidence suggest that corticalprojections tothe cerebellum may be more convergent. Here, we quantitatively compared anillustrative one-to-one model and amany-to-one convergent model, asking which provides amore accurate representationof cerebro-cerebellar connectivity. Using amulti-domainfMRIdataset (King et al. 2019), we built regularized linear regressionmodels using cerebral regionsas model features and thenused the weights from these models topredict cerebellar activitypatterns. We used arange of cortical parcellations as model features, resulting indivisions ofthe cortical surface for each hemisphere into7to2000regions. We compared twomodels ofcerebro-cerebellar connectivity:1) Winner-take-all which assumes aone-to-one mapping andkeeps the cortical regionwith the highest connectivity weight, setting all other

weights tozero. 2)Ridge regressionwhich assumes aconvergent many-to-one model and uses L2-normregularizationtoconstrainconnectivity weights tobe close tozero. The estimated connectivityweights from each model were used tomake predictions for cerebellar activity profiles inanoveltask set. The convergent “many-to-one”model outperformed the sparse “one-to-one”model forall cortical parcellations. As the granularity of the parcellationincreased, the predictive accuracyof the convergent model showed amodest increase. The opposite patternwas observed for thesparse one-to-one model, although the decrease inaccuracy with increasing granularity wasmore marked. We alsofound differences across the cerebellar cortex interms of predictiveaccuracy. Specifically, the one-to-one models performed best insensorimotor regions of thecerebellum (e.g lobules I-V), whereas the many-to-one model alsoperformed well incerebellarareas associated with more cognitive tasks (e.g., lobules VII), suggesting that aconvergentcerebro-cerebellar mapping is more likely for cognitive circuits. One limitationwith the currentanalyses is that all of the models underperformed relative tothe noise ceiling, demonstratingthat there is still potential for better predictive models. However, the results todate, derived fromarich set of functional data, challenges the view of one-to-one closed loopcircuitry as the corearchitectural feature of cortical-cerebellar connectivity.

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Poster

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

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Title: Machine Learning Pattern Recognition for Identifying and Detecting Spontaneous IPSCs

Authors: *E. WHITE[1], J. BRUNDAGE[2], H. WADSWORTH[3], C. MASON[3], D. HEDGES[5], J. T. YORGASON[4];
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Abstract: Analysis of patch clamp electrophysiology data can be a time consuming and error-prone task, with limited ways of controlling variation introduced during analysis. The analysis of spontaneously occurring events, such as spontaneous inhibitory post synaptic currents (sIPSCs), introduces an additional layer of complexity because these signals are typically smaller in amplitude, occur infrequently and can vary greatly from one laboratory to another. Many laboratories use a simple amplitude thresholding algorithm or signal matching tools for sIPSC

detection, and the detected events are then confirmed by a researcher. The present work developed and tested machine learning tools for sIPSC detection, with the goal of reducing error introduced through researcher input bias and computational time. Different model architectures and data input types are compared to determine which machine learning methods are best suited for the detection of sIPSCs. Manually defined sIPSCs were used as initial training sets (input features) for the created architectures. An XGBoost model—using standardized, raw waves as inputs—had the best performance for event detection, with an accuracy of 74.9% and an ROC-AUC of 0.82. Using feature reduction techniques, the number of input features for training was reduced from 4000 to 100 inputs with no drop in performance, thus lowering the computational burden of training. Finally, the trained model was validated in an analytic workflow, where it was used to identify and classify events in a new dataset. This validation demonstrated that a semi-automated approach using the machine learning powered peak detector was able to process raw recording data with comparable results to a manual workflow. To ensure the generalizability of this technique, the XGBoost model was given to various laboratories to implement on different types of data. The architecture's performance and analysis of different datasets proved to be successful and easy to use. Importantly, in all experiments, the machine learning detector was much quicker, with the manual method requiring an estimated 66 work hours to determine all peak locations, compared to just minutes for the machine learning method. These data indicate that an XGBoost model trained on standardized raw traces as inputs, rather than extracted features, is well suited for peak detection for sIPSCs and could greatly decrease analysis time and experimenter bias.

Disclosures: E. White: A. Employment/Salary (full or part-time):; Brigham Young University. **J. Brundage:** A. Employment/Salary (full or part-time):; Brigham Young University. **H. Wadsworth:** A. Employment/Salary (full or part-time):; Brigham Young University. **C. Mason:** A. Employment/Salary (full or part-time):; Brigham Young University. **D. Hedges:** A. Employment/Salary (full or part-time):; Billings Clinic. **J.T. Yorgason:** A. Employment/Salary (full or part-time):; Brigham Young University.

Poster

P966. Computational and Analysis Tools V

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P966.06

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

Support: NIH Grant K01MH120433

Title: Comparing Denoising Approaches in Ultra-High Field Resting State fMRI

Authors: *M. MEHTA[1,3], Y. JACOB[2], L. MORRIS[1];
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Imaging Inst., Icahn Sch. of Med. at Mount Sinai, new york, NY; [3]Dept. of Psychology, New York Univ., New York, NY

Abstract: Background: One of the biggest challenges in functional Magnetic Resonance Imaging (fMRI) research has been parsing brain activation (BOLD signal) from artifact noise. Multi-echo (ME) acquisition of fMRI data facilitates BOLD separation by capturing every slice at multiple echo times. The use of ME 7 Tesla data allows examination of more detailed signal at a high spatial resolution. Currently there are several mathematical approaches to combining and denoising ME data to obtain BOLD signal but the optimal method for high-resolution data is unknown.**Methods:** In this project, we aim to compare the predominant ME preprocessing pipelines: AFNI and MEICA. We also analyze individual echo images using SE AFNI preprocessing approach. We evaluate the quality of the denoised BOLD signal using global and regional temporal Signal-to-Noise Ratio (tSNR) and functional connectivity measures in healthy individuals (N=9).**Results:** Our preliminary data suggest that with minimal processing with smoothing a 6 mm full width at half maximum (FWHM) kernel in the MNI space, there was significant effect of combining and denoising method on the whole-brain tSNR [$F(7,63)=44.85$, $p<0.001$] and functional connectivity of key nodes of the default mode network (DMN) [$F(7, 63) = 10.59$, $p<0.001$], motor network [$F(7, 63)=2.68$, $p=0.017$] and reward network [$F(7, 63)=3.52$, $p=0.003$].**Conclusion:** Our preliminary results suggests that denoising and multi-echo combining approaches significantly impact the pre-processed BOLD signal. MEICA based denoising approach offers better tSNR, while AFNI based approaches seem better at conserving functional connectivity. However, visualization of seed-to-voxel connectivity showed presence of high but localized connectivity in the MEICA-based output. These results indicate a need for a global measure for signal quality of ME resting state (rs)-fMRI data.

Disclosures: M. Mehta: None. **Y. Jacob:** None. **L. Morris:** None.

Poster

P966. Computational and Analysis Tools V

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P966.07

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

Support: DoE ASCR

Title: Bayesian Inference in High-Dimensional Time-Series with the Orthogonal Stochastic Linear Mixing Model

Authors: *R. MENG, K. BOUCHARD;
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Abstract: Many modern neuroscience datasets are time-series that contain large numbers of output response variables sampled for prolonged periods of time. For example, the neurophysiology of 100s-1000's of neurons are recorded during behaviors and in response to sensory stimuli. While several latent-variable methods exist for analysis of such data, each has benefits and shortcomings from a computational or interpretational perspective. Within the class of Bayesian methods, an outstanding challenge is scalability of inference for datasets with large numbers of output responses and many samples.
We propose a new regression framework, the orthogonal stochastic linear mixing model (OSLMM), that enables inference in large time-series datasets. Multi-output Gaussian process models capture structure across multiple outputs, however they typically assume correlations between the output response variables are invariant in the input space. Stochastic linear mixing models assume the mixture coefficients depend on input, however, the inference is intractable for large datasets. Here, we propose the orthogonal stochastic linear mixing model (OSLMM) that introduces an orthogonal constraint amongst the mixing coefficients. This constraint reduces the computational burden of inference while retaining the capability to handle complex output dependence. We provide Markov chain Monte Carlo inference procedures for both SLMM and OSLMM. In neurophysiology recordings, we use the inferred latent functions for compact visualization of population responses to auditory stimuli, and demonstrate superior results compared to a competing method (GPFA). Specifically, compared to GPFA, OSLMM has better data-predictive performance (e.gcross-validated R2), and the structure of neural activity in the inferred latent states more accurately matches the parametric relationships of the amplitude and frequency of the stimuli. Thus, the interpretation of the latent variables is easier.
Together, these results demonstrate that OSLMM will be useful for the analysis of diverse, large-scale time-series datasets commonly found in neuroscience. More broadly, the results highlight the importance of continued development of methods to reveal latent structure in high-dimensional neuroscience datasets.

Disclosures: R. Meng: None. **K. Bouchard:** None.

Poster

P966. Computational and Analysis Tools V

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P966.08

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

Support: NSF DMS-1042134
NIH MH052090
NSF GRFP
KIBM IRG

Title: Modeling neuronal engagement in rhythmic network activity

Authors: *P. D. RIVIERE[1], G. SCHAMBERG[2], T. P. COLEMAN[3], L. M. RANGEL[4]; [1]Cognitive Sci., Univ. of California San Diego, La Jolla, CA; [2]Picower Inst. for Learning and Memory, MIT, Cambridge, MA; [3]Bioengineering, UCSD, La Jolla, CA; [4]Cognitive Sci., UCSD, San Diego, CA

Abstract: Neural spiking activity exhibits exquisite temporal structure that emerges from the interplay of various intrinsic and synaptic currents, requiring methods that can evaluate specific contributions to this structure from various possible neural influences. Spike timing is systematically constrained by stereotyped membrane responses and neuronal interactions, including refractory periods (milliseconds) during which neurons are unlikely or unable to fire action potentials, and rhythmic currents that create temporal windows of excitability (hundreds of milliseconds). Here, we offer a novel approach to (1) represent the relationship between neuronal spike timing and the phase of any network local field potential (LFP) oscillation, and (2) weigh the relative contributions of longer-timescale rhythmic constraints and shorter-timescale ones, such as the refractory period. Specifically, we use kernel density estimation to directly model the likelihood of observing particular phases when spikes occur, and subsequently compute the posterior probability of spiking over time given a phase time series. To model refractory constraints on neuronal spiking, we apply logistic regression to predict upcoming spiking from the immediately preceding spike history (3 ms). Within this logistic regression framework, we combine phase and short history information to generate a comprehensive model that explicitly tests the hypothesis that neurons are engaged in a given rhythmic network process, while still accounting for intrinsic refractory constraints on spiking. We compare this comprehensive model against models that capture long-term (250 ms) autocorrelations in spike trains, in order to assess whether temporal structure in spike trains is in fact predominantly related to network dynamics reflected in the LFP, rather than a variety of alternative influences that would contribute to autocorrelations (e.g. spike-frequency adaptation, alternative rhythmic modulation). We first validate the models in simulated neurons, and subsequently apply the models to assess the relationship between hippocampal CA1 inhibitory interneuron spiking and the phase of the theta (4-12 Hz) rhythm. For most interneurons, models that combined immediate history (capturing refractory periods) and phase (reflecting rhythmic current influences) most accurately described the temporal structure of neuronal spike trains. With this modeling suite, we model variability in spike trains, and specifically test the relationship between network-level rhythmic currents and neuronal spike timing.

Disclosures: P.D. Riviere: None. **G. Schamberg:** None. **T.P. Coleman:** None. **L.M. Rangel:** None.

Poster

P966. Computational and Analysis Tools V

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

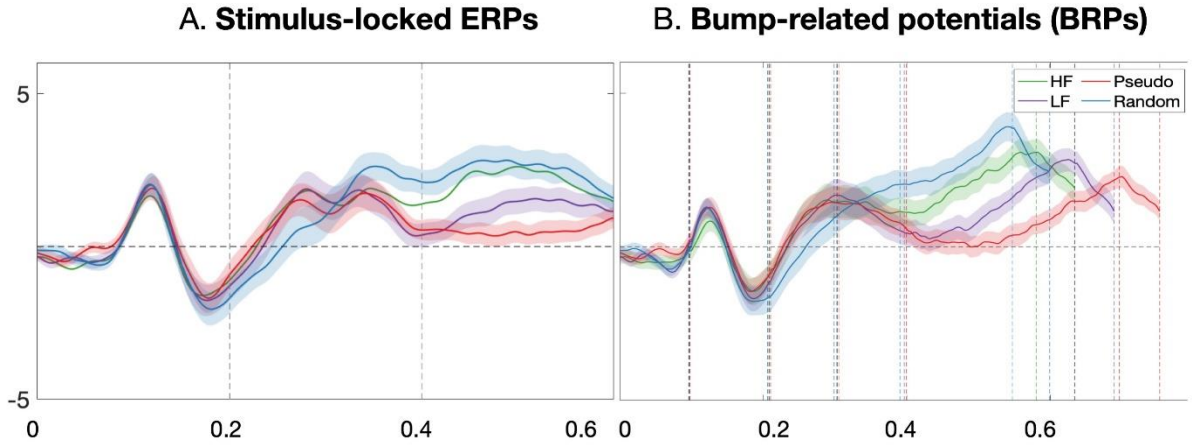
Program #/Poster #: P966.09

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

Title: A novel method to account for temporal variability in EEG: Bump-related potentials

Authors: *H. S. BERBERYAN[1], H. VAN RIJN[2], J. P. BORST[1];
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Abstract: Over the past century, different recording techniques have been used to measure brain activity. One of these techniques, electroencephalography (EEG), has the undoubted advantage of a very high temporal resolution that allows for recording brain activity at a millisecond timescale. This advantage, however, also presents a challenge in the analysis because cognitive processes are not aligned precisely between participants, and between trials within a participant. When applying the traditional event-related potential technique – that is, averaging the signal over trials and participants – part of the information that is present in single trials is lost due to the averaging procedure. In particular further away from a fixed time point such as stimulus or response, the average signal tends to become blurry and uninformative. To overcome this limitation, we applied a novel machine learning algorithm called Hidden semi-Markov Model multivariate pattern analysis (HsMM-MVPA; Anderson, Zhang, Borst, & Walsh, 2016). This algorithm was developed to identify processing stages on a trial-by-trial basis by searching for the peaks across the scalp (so-called bumps) that signify critical events. In the current project, the results of HsMM-MVPA were applied to calculate bump-related potentials (BRPs). BRPs are extensions of classical ERP analysis that are calculated by aligning EEG data to the locations of the peaks identified with HsMM-MVPA. BRPs were calculated on data from a lexical decision task in which the frequency of words (high frequency and low frequency) and "wordness" of non-words (pseudo words and random letter strings) were varied (Berberyan, van Rijn, Borst, 2021). The results of analysis revealed clear early peaks present in both ERPs (Figure 1A) and BRPs (Figure 1B). While in ERPs the information from about 400 ms was less clear, BRPs could successfully recover the precise location of the later peaks for each condition. Taken together, the results suggest that BRP technique is a promising approach for gaining new insights into cognitive processing.



Disclosures: H.S. Berberyan: None. **H. Van Rijn:** None. **J.P. Borst:** None.

Poster

P966. Computational and Analysis Tools V

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:15 PM - 2:15 PM

Program #/Poster #: P966.10

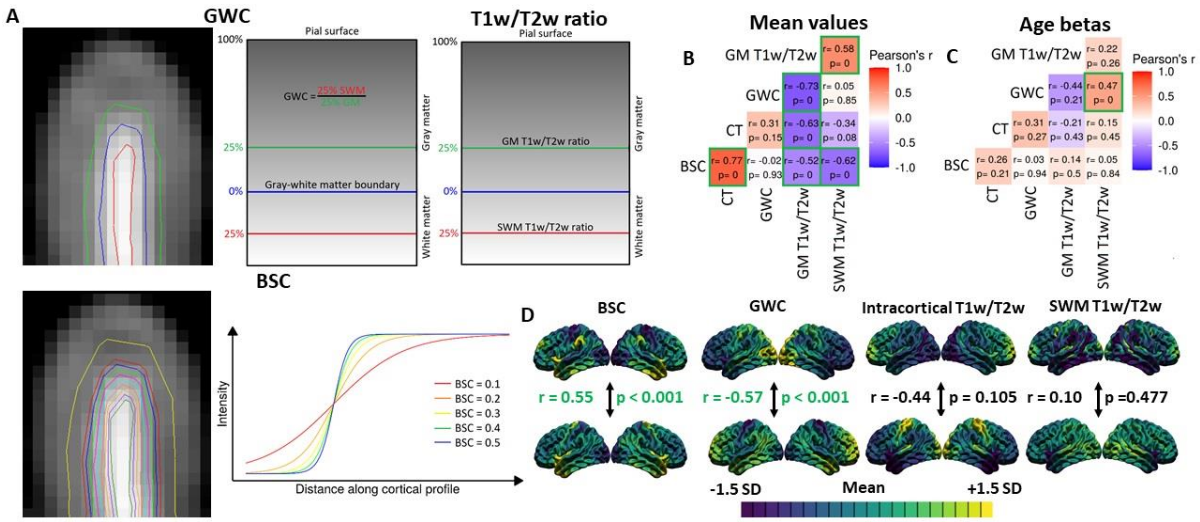
Topic: I.06. Computation, Modeling, and Simulation

Title: Comparing age trajectories of MRI cortical markers and myelin: An exploratory study

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Abstract: The biological interpretations of neuroanatomical magnetic resonance imaging (MRI) are challenging. While MRI indices such as cortical thickness (CT) relate to macrostructural organization of the cortical mantle, there is evidence that CT estimates are impacted by cortical myelin and other microstructural properties. Here, we compare the spatial organization and age effects of CT to 3 measures of cortical microstructure (gray-white matter contrast [GWC], the newly developed boundary sharpness coefficient [BSC] from our group, gray matter [GM] T1w/T2w ratio and superficial white matter [SWM] T1w/T2w ratio), and probe their specificity to cellular organization using the histological reconstruction of BigBrain.
T1-weighted MPRAGE (1 mm3 voxels) and T2-weighted SPACE (0.64 mm3 voxels) images from 127 healthy subjects (aged 18-81, 76F, 51M) were used. Cortical surfaces were generated and CT estimates were derived using the CIVET 2.1.0 pipeline (see Fig 1A for derivation of microstructural metrics). For each marker and vertex, the mean value across subjects was computed and a linear model with age and sex as predictors was fitted. The correlation of the surface maps was assessed and significance tested via spin tests.
Results included a significant spatial relationship of the mean values between the GM T1w/T2w ratio and the GWC, BSC and CT, and between SWM T1w/T2w ratio and the BSC (Fig 1B). There was a positive spatial correlation between the age effect of the GWC and SWM T1w/T2w ratio (Fig 1C). A significant relationship was found between the BSC and GWC calculated on BigBrain and their MRI equivalent, although it is likely that the BSC correlation is mediated by CT (Fig 1D).
Our finding of spatial correlations being higher for mean values than for age effect indicates that

some measures tend to covary at the cortex-wide level, but age trajectories are likely influenced by different interactions of microstructural changes. Our BigBrain results indicate a general trend of GM T1w signal and myelin being inversely related to the density of cells, although that relationship is weak to moderate.



A. The GWC was calculated by dividing the intensity of the T1w image at 25% of CT translated in SWM by the intensity at 25% of CT in GM. The BSC was defined as the growth parameter of a sigmoid curve fitted to 10 intensities sampled across the gray-white matter boundary. A sharper gray-white matter transition is reflected by a high BSC value. GM T1w/T2w ratio was sampled at 25% of CT in GM, and SWM T1w/T2w ratio was sampled at 25% of CT translated in SWM. B. Correlations between cortical maps of the mean values between the markers. P-values are corrected for false discovery rate. Correlations at p<0.05 are highlighted in green. C. Correlations between cortical maps of the betas of the age term between the markers. D. Correlations between cortical maps of the markers computed on BigBrain (top row) and markers computed on MRI images (bottom row)*.
* The T1w/T2w markers on MRI images are compared with the intensities on BigBrain at the same sampling distance

Disclosures: O. Parent: None. **E. Olafson:** None. **A. Bussy:** None. **S. Tullo:** None. **A. Salaciak:** None. **S. Farzin:** None. **S.A. Bedford:** None. **M. Béland:** None. **C.L. Tardif:** None. **G.A. Devenyi:** None. **M. Chakravarty:** None.

Poster

P057. Angelman and Other Developmental Disorders

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P057.01

Topic: A.07. Developmental Disorders

Support: NIDCD Grant R21 DC01646

Title: Temporal profile of genomic imprinting of the E6-AP ubiquitin ligase (Ube3a) gene in developing GABAergic neurons in vivo

Authors: *K. GUTHRIE[1], S. MIRI[2], M. AMIR[2], A. VISAVADIYA[2];
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Abstract: Angelman Syndrome (AS) is a severe neurodevelopmental disorder characterized by developmental delay, intellectual disability, seizures, lack of speech, and motor impairment. AS is caused by maternally inherited deletions/mutations in the gene encoding the HECT E3 ubiquitin ligase Ube3a. This eliminates functional Ube3a during early brain development as the paternal Ube3a allele undergoes epigenetic silencing in neurons. In AS model mice, almost all neuronal Ube3a protein is lost by birth. Exceptions include immature neurons born during late fetal stages, which retain some paternal Ube3a expression, and progenitor cells in forebrain neurogenic niches. Differential splicing generates multiple Ube3a isoforms with distinct patterns of intracellular localization in neurons (nuclear vs cytosolic) thought to reflect multiple functions. Shifts in localization occur with increasing neuron maturation in vitro, and during early postnatal development. However, a complete profile of Ube3a expression and paternal silencing during neuron development in vivo, from cell birth, neuroblast migration and later maturation, has yet to be characterized. Using adult AS model mice, and adult mice expressing Ube3a-YFP from the paternal allele, we tracked the expression and localization of Ube3a in SVZ-generated GABAergic olfactory granule cells over the course of their development using bromodeoxyuridine (BrdU) and markers indicative of maturational stage. We found that SVZ progenitors and migrating neuroblasts expressed Ube3a in AS and wild-type (WT) mice, and Ube3a-YFP in this strain. In these populations, Ube3a localization was predominantly cytosolic. As they matured in the olfactory bulb, transitioning from doublecortin to new NeuN expression while extending an apical process at 7-9 days post BrdU, Ube3a and Ube3a-YFP shifted to nuclear localization, with cytosolic Ube3a maintained. Some BrdU+ neurons exhibited nuclear NeuN and Ube3a colocalization in both WT and AS mice, with Ube3a disappearing from nuclei in AS neurons rapidly thereafter. Similarly, Ube3a-YFP disappeared soon after the shift to the nucleus, indicating that at this early developmental stage, potential nuclear functions of Ube3a isoform 3, which predominates in this compartment, are permanently lost from AS neurons. Quantitative analysis of labeled granule cell counts in WT mice at 14 days post-BrdU confirmed this as 93.8% of Brdu+ cells were also NeuN+/Ube3a+, whereas in AS mice, 2.2% of BrdU+ cells were Ube3a+, with 93.8% of cells BrdU+/NeuN+ only. In contrast to a report of disrupted adult hippocampal neurogenesis in AS mice, we found no evidence of altered olfactory neurogenesis.

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Poster

P057. Angelman and Other Developmental Disorders

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P057.02

Topic: A.07. Developmental Disorders

Support: Taysha Gene Therapies, Inc.

Title: RNA interference to treat neurological genetic disorders

Authors: *R. K. BUTLER[1], H. KANG[2], V. ZARIC[1], A. RAHIM[1], S. P. DEVRIES[1], Y. MA[1], S. J. GRAY[2];
[1]Psychiatry, [2]Pediatrics, Univ. of Texas Southwestern Med. Ctr., Dallas, TX

Abstract: RNA interference could potentially treat numerous genetic disorders by indirectly elevating healthy protein levels genotypically present on a silenced allele or on a duplicated gene. Deficits in neuronal UBE3A protein result in Angelman Syndrome (AS). UBE3A is regulated through genetic imprinting whereby the maternal allele is expressed and the paternal allele is silenced. Paternal silencing is induced with a portion of a large, non-coding antisense transcript specific to UBE3A (UBE3A-ATS). Previously it has been demonstrated that anti-sense oligonucleotides targeting the region between SNORD115 and upstream of UBE3A-ATS elevate paternal expression of UBE3A and improve behavior deficits in an AS mouse model. Using a similar transgenic model, targeting SNORD115 with CRISPR/Cas9 also elevated UBE3A and improved behavior deficits. We explored whether small interfering RNAs (siRNA) targeting the region encompassing SNORD115 and upstream of UBE3A-ATS elevated UBE3A transcript in human cultured neurons. Furthermore, we administered intrathecally at P7-P10 an adeno-associated virus type 9 (AAV9) with a short-hairpin RNA (shRNA) targeting the same transcript region in the AS mouse model. *In vitro* screens (N=3) of 46 siRNA candidates (10 nM; 24 hour incubation) indicate that several candidates reduce UBE3A-ATS and increase UBE3A in human cultured M17 neuroblastoma cells. Preliminary data suggest that shRNA/AAV9 delivered intrathecally induces paternal expression of UBE3A in Purkinje cells of the cerebellum and rescues nest building behavior deficits in the AS mouse model at 8 and 12-weeks post-injection. No sex differences were observed. Similar siRNA and shRNA/AAV9 approaches are being performed concurrently in human cells and animal models of Prader-Willi Syndrome and Spinal Muscular Atrophy. Taken together, this study demonstrates the potential for a vector-mediated and permanent RNA interference approach to treat neurological disorders.

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Poster

P057. Angelman and Other Developmental Disorders

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P057.03

Topic: A.07. Developmental Disorders

Support: NIH HG006687
NIH R00MH117165

Title: Genome-wide significant identification of novel neurodevelopmental candidate proteins through a two-stage CLUMP analysis workflow

Authors: T. M. AKINWE, E. MEHINOVIC, J. K. NG, T. N. TURNER;
Washington Univ. in St. Louis, St. Louis, MO

Abstract: Genes associated with neurodevelopmental disorders (NDDs) have been previously identified with an excess of protein-coding *de novo* variants (DNVs). Most of the emphasis thus far has been on likely gene-disruptive DNVs. Missense DNVs, have been less studied because their functional consequence on protein function is more difficult to interpret. Nonetheless, there have been efforts made to look at rare missense variants in high-impact risk genes and observe functional effects to the encoded protein. One area that has been examined is whether missense variants are present in a domain, as presence in a domain may have a more severe impact. However, the assumption that rare missense mutations of large effect will only occur in protein domains may obscure functionally important regions outside of known domains. To assess clustering of mutations in an unbiased manner, we use the CLUstering by Mutation Position (CLUMP) algorithm. CLUMP has previously been applied to rare missense NDD-associated variants., but we now present an updated two-stage workflow that allows CLUMP to test for genome-wide significant (GWS) clustering. Using CLUMP, we tested 45,221 protein-coding DNVs within 13,353 proteins (18,762 genes) from 31,058 NDD families against two negative controls: the 1000 Genomes Project (1000G, n = 670) and the Genome Aggregation Database (gnomAD, n = 76,156). We began with Phase I where we applied a low number of permutations (z = 100), and proteins that met nominal significance ($p < 0.01$) proceeded to Phase II at a higher permutation ($z = p < 1 \times 10\text{-}7$). Proteins that reached multiple-test correction significance ($p < 2.6 \times 10\text{-}6$) had GWS mutation clusters. After generating CLUMP results, we visualized clusters and compared them to previously mapped protein domains and regions using 3D protein structure analysis. We identified three GWS CLUMPed proteins (GNA01, PPP1CB, CREBBP) in comparison to the 1000G dataset. CLUMP analysis with gnomAD as a control dataset is underway, but we have identified four significant proteins (GNAO1, PSMC5, DDX23, and COG4), thus far. By updating the CLUMP workflow, we have been able to find six GWS CLUMPed proteins, two of which (PSMC5 and DDX23) are new candidate proteins that require functional assessment beyond the *in silico* approach. Overall, CLUMP allows for a method to assess clustering of rare missense mutations beyond the context of established protein domains. Significant mutation clusters can be further analyzed to identify new functional or therapeutic targets for NDDs.

Disclosures: T.M. Akinwe: None. **E. Mehinovic:** None. **J.K. Ng:** None. **T.N. Turner:** None.

Poster

P057. Angelman and Other Developmental Disorders

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Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P057.04

Topic: A.07. Developmental Disorders

Support: The Pitt Hopkins Research Foundation

Title: MECP2 gene replacement therapy shows efficacy in patient cell lines and a mouse model of Pitt Hopkins Syndrome

Authors: *C. DENNYS[1], K. RICH[2], J. SIERRA DELGADI[1], J. ZHANG[1], P. ANDRES CORNEJO[3], P. FRANCISCO FERNANDEZ[3], S. POWERS[1], R. RODRIGO[2], A. HARTLAUB[1], R. PINEDA[1], P. COGRAM[3], K. C. MEYER[4];
[1]Nationwide Children's Hosp., Columbus, OH; [2]The Ohio State Univ., Columbus, OH; [3]Univ. of Chile, Santiago, Chile; [4]Ctr. for Genetherapy, Res. Inst. Nationwide Childrens Hosp., Columbus, OH

Abstract: Pitt Hopkins Syndrome (PTHS) is a neurological disorder caused by mutations in the TCF4 gene leading to haploinsufficiency affecting 1 in 34,000 to 41,000 individuals. Patients present with developmental delays, intellectual disabilities, microcephaly and seizures along with a broad spectrum of behavioral symptoms. Unfortunately, the large size and number of different isoforms of TCF4 limits the use of gene replacement therapy for the treatment of PTHS. Furthermore, while neurons and oligodendrocytes are heavily implicated in disease pathology, little research has been performed on other non-cell autonomous contributions. Thus, identifying the roles of other cell types, such as astrocytes, in disease pathology is critical as gene therapy approaches must target disease relevant cell types. We have previously developed a rapid reprogramming method to convert patient skin fibroblasts to neuronal progenitor cells (NPCs). These cells can subsequently be differentiated into induced astrocytes (iAs) to investigate the contributions of iAs to PTHS. To evaluate the impact of TCF4 mutations on fibroblasts, NPCs, and iAs we used western blot, co-cultures, qPCR and immunostaining. Co-culture analysis demonstrated that PTHS iAs have a negative impact on neuron morphology and survival. Interestingly, we also observed that NPCs with deletion mutations have difficulty differentiating into iAs. Further proteomic analysis identified down regulation of MeCP2 protein levels in these patient lines. AAV9 mediated restoration of MECP2 in NPCs containing a TCF4 deletion restored differentiation defects previously observed. Given that TCF4 mutations are known to cause aberrant differentiation, these findings suggest that an MECP2 gene therapy approach may be an effective treatment strategy for PTHS. To evaluate this, we injected AAV9.MECP2 intracerebroventricularly (ICV) to P0-P1 TCF4 knockout mice. Behavioral analysis indicated amelioration of the aberrant phenotype in AAV9.MECP2 injected mice demonstrating that

MECP2 gene therapy might be a promising therapeutic strategy for PTHS. Combined, for the first time, this data demonstrates that astrocytes play a critical role in PTHS disease pathology. Furthermore, current limitations to PTHS gene therapy may be circumvented by utilizing an alternative, MECP2 gene replacement approach. Thus, we have discovered a new disease mechanism for PTHS in addition to identifying a novel therapeutic approach.

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Poster

P057. Angelman and Other Developmental Disorders

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P057.05

Topic: A.07. Developmental Disorders

Support: Foundation for Prader-Willi Research Pilot Grant

Title: Molecular changes in Prader-Willi syndrome neurons reveals clues about increased autism susceptibility.

Authors: *A. VICTOR[1], M. DONALDSON[2], D. JOHNSON[3], W. MILLER[3], L. T. REITER[4]; [1]Univ. of Tennessee Hlth. Sci. C Neurosci. Grad. Program, Memphis, TN; [2]Dept. of Pediatric Dent., [3]Mol. Bioinformatics Core, [4]Neurol., Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Prader-Willi syndrome (PWS) is a neurodevelopmental disorder characterized by hormonal dysregulation, obesity, intellectual disability, and behavioral problems. Most PWS cases are caused by paternal interstitial deletions of 15q11.2-q13, while a smaller number of cases are caused by maternal uniparental disomy (UPD), where only maternal copies of the PWS critical region are present. The PWS critical region is imprinted, meaning certain genes on the maternal chromosome are silent in neurons, resulting in a phenotype that is milder than, but equivalent to PWS cases where the paternal copies are deleted. There are, however, notable phenotypic differences between PWS Deletion (PW-del) and PWS UPD (PW-UPD). PW-UPD cases show milder phenotypes but are associated with an increased risk of developing autism spectrum disorder (ASD) and cycloid psychosis. Understanding the molecular differences

between PW-del versus UPD cases may provide clues to this increased risk for ASD. We have established a large collection of dental pulp stem cell (DPSC) lines derived from the teeth of subjects with various neurogenetic disorders. We regularly differentiate these stem cell lines into cortical-like neuronal cultures. Using neuronal cultures derived directly from DPSC of affected individuals and neurotypical controls, we preformed RNAseq studies to investigate differential gene expression changes associated with PWS subtypes and increased ASD risk. We found key molecular differences between PW-del and UPD both within and outside of the 15q region. In addition to genes that are specific to each class, we found 9 transcripts outside of the 15q critical region that may contribute to a core PWS phenotype, including *SNTB2*, which is involved in the regulation of secretory granules. In the PW-UPD +ASD group, we found 380 transcripts significantly differentially expressed versus the -ASD groups. Enrichment analysis revealed a strong decrease in mitochondrial-associated transcripts in this transcript set. Immunocytochemistry revealed not only a significant reduction in mitochondrial volume, but also a mitochondrial aggregation phenotype in the UPD +ASD neurons. Decreased mitochondrial metabolism in PWS neurons may cause changes in synaptic function that underlie the increased risk for ASD in PW-UPD individuals.

Disclosures: A. Victor: None. **M. Donaldson:** None. **D. Johnson:** None. **W. Miller:** None. **L.T. Reiter:** None.

Poster

P057. Angelman and Other Developmental Disorders

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P057.06

Topic: A.07. Developmental Disorders

Support: PI20/00773

Title: Effect of the combination of β-caryophyllene and cannabidiol in a murine model of Dravet syndrome

Authors: C. ALONSO[1,2,3], V. SATTA[1,2,3], I. HERNÁNDEZ[4], J. FERNÁNDEZ-RUIZ[1,2,3], ***O. SAGREDO**[1,2,3];
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Abstract: Dravet syndrome (DS) is a genetic epileptic encephalopathy usually caused by mutations in the Scn1a gene, which encodes the α1 subunit of the voltage-gated sodium channel

$Na_V1.1$. Clinically, patients are affected by multiple types of seizures that begin at very early ages of development and generate several behavioural comorbidities that accompany individuals throughout their lives. Patients are currently resistant to treatment with antiepileptic drugs, and lack of efficacious disease-modifying therapies. Recent clinical evidences have demonstrated that the cannabidiol (CBD), formulated and licenced as Epidiolex, has potential to reduce seizure frequency and intensity in these patients, and might serve as a disease modifier too. The objective of this study was to investigate whether β-caryophyllene (BCP), a terpenoid with a broad-spectrum profile abundantly found in the *Cannabis sativa* plant, is also a good candidate both for controlling seizures and for attenuating/delaying the progression of associated comorbidities. As a second objective, we also investigated the potential of the combination of CBD and BCP to improve the therapeutic management in DS. To this end, we used a new experimental model of DS, which consisted of heterozygous conditional knock-in mice carrying a missense mutation (A1783V) in *Scn1a* gene expressed exclusively in neurons of the CNS (Syn-Cre/Scn1a$^{WT/A1783V}$), using two different experimental approaches. In a first approach, we evaluated the anticonvulsant effect of BCP against the proconvulsant agent pentylenetetrazol (PTZ). Our data proved that Syn-Cre/Scn1a$^{WT/A1783V}$ mice are more susceptible than wildtype animals to PTZ, whereas BCP reduced its proconvulsant activity in both genotypes. In a second approach, we investigated the effect of a chronic treatment with BCP, CBD and their combination on various behavioural comorbidities associated with the progression of DS in Syn-Cre/Scn1a$^{WT/A1783V}$ mice. The three treatments corrected the behavioural alterations observed in these mice and delayed their premature mortality, with the combined treatment being the most effective. The reactive astrogliosis and observed in the prefrontal cortex and the hippocampal dentate gyrus of Syn-Cre/Scn1a$^{WT/A1783V}$ mice was also reduced after the three treatments and once again the combined treatment was the best option. In conclusion, this study confirms the potential of CBD as a disease modifier in DS, proposes an additional phytocannabinoid, BCP, as an alternative symptom-alleviating and disease-modifying agent, and provides the first evidence that their combination may be a promising therapeutic strategy for the treatment of DS.

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Poster

P057. Angelman and Other Developmental Disorders

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Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P057.07

Topic: A.07. Developmental Disorders

Support: Angelman Syndrome Alliance

Title: Delta EEG rhythms are increased in Angelman syndrome during overnight sleep

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Abstract: Angelman syndrome (AS) is a neurodevelopmental disorder resulting from decreased expression of the maternal copy of the paternally imprinted *UBE3A* gene on chromosome 15. AS is characterized by intellectual disability, impaired speech and motor skills, and sleep abnormalities but currently lacks any treatment. However, mouse models have shown that unsilencing the dormant paternal copy of *Ube3a* has been an effective mechanism to restore the functionality of the UBE3A protein, thus clinical trials using this approach are on the near horizon. Developing biomarkers is essential for assessing responses to treatment when clinical trials begin, and our prior work quantifying clinical EEGs has defined promising biomarkers for AS. Here, we sought to extend analyses to periods of overnight sleep, as sleep impairments affect ~90% of individuals with AS. We retrospectively analyzed twelve overnight sleep studies from patients with AS with age and sex matched Down syndrome and neurotypical controls. We specifically examined low-frequency (2-4 Hz) delta rhythms and sleep spindles during NREM sleep. Delta rhythms were increased during overnight sleep in AS, and the magnitude of delta abnormalities was comparable between periods of wake and periods of sleep. There were no changes in the number or duration of sleep spindles in AS, but spindles did occur at slightly higher frequencies. Overall, we conclude that periods of wakefulness are sufficient, and perhaps ideal, to quantify delta rhythms for use as biomarkers.

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Poster

P057. Angelman and Other Developmental Disorders

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Program #/Poster #: P057.08

Topic: A.07. Developmental Disorders

Support: NIH 3R34DA050255
NIH R01DA043678
NIH R01DA042988

Title: Functional and structural differences in the brains of one-year-old infants with Turner Syndrome in comparison to typically developing children

Authors: *R. BLANCHETT[1], H. CHEN[2], E. CORNEA[3], M. DAVENPORT[4], D. REINHARTSEN[5], M. DERAMUS[5], R. PRETZEL[5], J. H. GILMORE[7], S. HOOPER[6], M. A. STYNER[8], W. GAO[9], R. KNICKMEYER[1];

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Abstract: Turner syndrome (TS), or X monosomy, is often accompanied by deficits in executive function, visuospatial reasoning, and social cognition. Magnetic resonance imaging studies of adults and school-age children with TS suggest these deficits may reflect differences in anatomical and functional connectivity. However, no imaging studies have explored anatomical and functional connectivity in infants with TS. Consequently, it is unclear at what point in development connectivity differences emerge. To address this gap, we compared functional and structural connectivity of one-year-old infants with TS to typically developing boys and girls of the same age. We examined functional connectivity between the right precentral gyrus and the following regions: right and left calcarine cortex, right and left lingual cortex, and right supramarginal cortex. Regions were selected because they show reduced volume in 1-year old infants with TS compared to either male or female controls. To assess anatomical connectivity, we examined diffusivity indices along the superior longitudinal fasciculus (SLF). We focused on the SLF because DTI studies of adults and school-age children with TS have revealed differences in white matter microstructure of this tract. An exploratory analysis of 31 additional white matter tracts was also performed. TS and control groups did not differ in radial, axial, and mean diffusivity, or fractional anisotropy along the SLF. However, exploratory analyses revealed significant group differences in axial, radial, and mean diffusivity along the temporoparietal segments of the left and right arcuate fasciculi. Results indicate that some connectivity differences observed later in development of TS individuals are not present at one year, suggesting early intervention could prevent some of these differences and, by extension, some of the cognitive challenges associated with TS. However, significant differences in the left and right arcuate fasciculi appear to arise very early in development and could have implications for cognitive processes related to these tracts including auditory spatial attention, visuospatial cognition, and mathematical operations.

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Poster

P057. Angelman and Other Developmental Disorders

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Program #/Poster #: P057.09

Topic: A.07. Developmental Disorders

Support: R01 MH120174
R01 MH119185
U01MH119738

Title: Using automated, objective facial measurements to characterize facial dysmorphology as a potential risk factors in psychosis.

Authors: *M. KRALL[1], D. R. ROALF[2], J. JEE[3], D. MCDONALD-MCGINN[4], R. T. SHINOHARA[1], L. WHITE[4], E. MCCLELLAN[4], A. CROW[1], T. B. CROWLEY[4], A. BAILEY[4], R. S. GALLAGHER[4], V. GONZENBACH[1], P. J. MOBERG[1], B. I. TURETSKY[1], C. KOHLER[1], R. C. GUR[5], E. CHEN[4], R. E. GUR[1], K. GAISER[1], D. MCGINN[1]; [2]Neuropsychiatry, [1]Univ. of Pennsylvania, Philadelphia, PA; [3]Univ. of Pennslyvania, Philadelphia, PA; [4]Children's Hosp. of Philadelphia, Philadelphia, PA; [5]Univ. Pennsylvania, Philadelphia, PA

Abstract: Background. Psychosis is a brain disorder that typically develops during adolescence or young adulthood and 22q11.2 deletion syndrome (22q11DS) is a chromosomal deletion that is highly associated with psychosis-spectrum (PS) disorders. Individuals with 22q11DS are known to have facial dysmorphology while these features have been documented in individuals with schizophrenia, which are likely due to disruptions of prenatal development. These minor physical abnormalities (MPA) of the face may be informative in understanding the nature and timing of risk factors for brain disorders like psychosis. Thus, the aim of the current study is to compare facial features among four groups (22q11DS; schizophrenia (SZ); clinical high risk for psychosis (CHR); and healthy individuals) to determine whether people with psychosis spectrum disorders and 22q11DS exhibit similar MPAs. **Methods.** 2D facial images were collected from patients with 22q11DS (n=171), SZ (n=37), CHR (n=16) and HC (n=94). Images were analyzed using the software Emotrics, a newly developed, machine-learning algorithm that provides automated, objective facial measurements using 68 facial coordinates that are automatically placed. Facial landmarking in Emotrics outlines the superior border of the brow, the free margin of the upper and lower eyelids, the nasal midline, the nasal base, the mucosal edge and vermillion-cutaneous junction of the upper and lower lips, and the lower two-thirds of the face. The outcome measures from Emotrics included eyebrow height, marginal reflex distance (MRD), and palpebral fissure (PFH). **Results.** As expected, we found that across all features, those with 22q11DS differed the most from the HC group, however SZ and CHR showed similar patterns as those with 22q11DS. As compared to HC, 22q11DS and SZ showed smaller MRD ($p<0.001$) and PFH ($p<0.001$), while CHR only showed lower MRD ($p<0.008$). Specificity analysis indicated that MRD differences were most prominent from the pupil to top eyelid. There were no differences in brow height. **Conclusion** These preliminary results suggest overlap in facial dysmorphogenesis between 22q11DS and psychosis. Results are promising and suggest that individuals with psychosis exhibit a similar pattern of facial MPAs to those with 22q11DS, which may help understand the developmental basis for this complex brain disorder.

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Poster

P059. Animal Models of Developmental Disorders

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P059.01

Topic: H.13. Schizophrenia

Support: Fellowship 725800 for LAM from Consejo Nacional de Ciencia y Tecnología, México

Title: Differential expression of plasticity of the medial and lateral perforant path input to the dentate gyrus in a neurodevelopment model of schizophrenia

Authors: *L. A. MÁRQUEZ, C. LÓPEZ-RUBALCAVA, E. J. GALVÁN;
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Abstract: Differential expression of plasticity of the medial and lateral perforant path input to the dentate gyrus in a neurodevelopment model of schizophrenia
Transient hypofunction of the NMDA receptor-mediated synaptic transmission during early postnatal development induces schizophrenia-like behavior. Despite the growing effort to understand the role of the hippocampus on the pathophysiology of schizophrenia, little is known about alterations in the synaptic transmission conveyed by the entorhinal cortex and the dentate gyrus (DG). We performed extracellular recordings (fEPSPs) on juvenile acute hippocampal slices (P30-P35) to explore alterations in synaptic transmission of the medial perforant path (MPP) and lateral perforant path (LPP) to the DG in animals treated with MK-801 (0.2 mg/kg s.c; P7-P11). We found that synaptic strength is decreased in the MPP – DG synapse, whereas the opposite occurs in the LPP – DG synapse. When comparing the paired-pulse ratio (PPR), we found that only LPP-DG synapses of MK-801 slices showed a reduction in neurotransmitter release probability. In addition, theta-burst stimulation failed to induce stable LTP in the LPP – DG synapse and induced a blunted response in the MPP – DG synapse of the MK-801 treated animals, indicating that the somatosensorial processing of the DG (spatial information for MPP and contextual information for LPP) exhibit dysregulation and likely, cognitive impairment. Therefore, we evaluated the performance of MK-801 treated rats in the Barnes maze, a hippocampus-dependent task. Compared to control, the MK-801 treated animals exhibited impaired performance. Collectively, these results indicate altered information transfer from the entorhinal cortex to DG that is reflected in altered synaptic transmission and impaired DG-related cognitive capabilities.

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Poster

P059. Animal Models of Developmental Disorders

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Program #/Poster #: P059.02

Topic: H.13. Schizophrenia

Support: NIH 5R01MH096274-08, and a BBRF Narsad YI 2019
NINDS intramural research program (ZIA NS003168)

Title: Selective GSK3α and β inhibition alters working memory-related behavior in a mouse model of 22q11.2 deletion syndrome

Authors: *C. M. ALOIMONOS[1], J. PASSECKER[2,1,3], C.-Y. CHANG[3], A. DAGUNTS[1], D. A. KUPFERSCHMIDT[1], J. A. GOGOS[3], J. A. GORDON[4];
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Abstract: Overactivation of the Glycogen Synthase Kinase (GSK3) pathway has been implicated in a variety of neurological and psychiatric disorders. Non-selective GSK3 (α and β isoform) inhibitors have been found to rescue cognitive deficits in a variety of preclinical models, but their non-selective profiles hamper their preclinical research utility. Recently developed selective inhibitors allow targeted inhibition of each isoform, but their impacts on cognition and disease-relevant cognitive dysfunction are unresolved. Here, we tested their potential to rescue spatial working memory deficits in the *Df(16)A*$^{+/-}$ mouse model relevant to the 22q11.2 deletion syndrome. The selective GSK3β inhibitor BRD3731 administered in early postnatal development (P7-P28) rescues acquisition deficits in spatial working memory in male but not female *Df(16)A*$^{+/-}$ mice (n = 121, p=0.01, 3-way ANOVA). GSK3β inhibition also rescued deficits in theta frequency (4-12 Hz) coherence between ventral hippocampus and medial prefrontal cortex, a neurophysiological correlate of spatial working memory performance, in *Df(16)A*$^{+/-}$ mice (n=98, p=0.02, 3-way ANOVA). By contrast, selective postnatal GSK3α inhibition with BRD0705 failed to rescue task acquisition deficits, while it did reverse deficits in task performance under conditions of increased memory demand in both male and female *Df(16)A*$^{+/-}$ mice (n=109, p=0.003, 3-way ANOVA). Detailed behavioral tracking revealed no evidence of differences in locomotor behavior that could explain these effects. Overall, this work indicates differential roles of GSK3α and β isoforms in the development of the neural circuits that support spatial working memory and its disease-relevant dysfunction in mice. Ongoing transcriptomic analysis of the medial prefrontal cortex and ventral hippocampus of wildtype and *Df(16)A*$^{+/-}$ mice at various developmental stages stands to inform the molecular basis of our behavioral and neurophysiological findings.

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Poster

P059. Animal Models of Developmental Disorders

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Program #/Poster #: P059.03

Topic: H.13. Schizophrenia

Support: NINDS Intramural Research Program ZIA NS003168
NIGMS FI2GM133387

Title: Alterations in neural circuit function during working memory task acquisition in Setd1a-deficient mice

Authors: *M. J. RAILING[1], S. HUPALO[1,2], M. V. MYROSHNYCHENKO[1], J. A. GOGOS[3], D. A. KUPFERSCHMIDT[1], J. A. GORDON[4,1];
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Abstract: Heterozygous null mutations of *SETD1A* are definitively linked to an increase in schizophrenia risk. Mice carrying a heterozygous loss-of-function mutation in *Setd1a* have been shown to exhibit deficits in working memory, a cognitive function disrupted in schizophrenia. To explore the neurobiological consequences of SETD1A haploinsufficiency, we examined connectivity within a circuit including the ventral hippocampus (vHPC), medial prefrontal cortex (mPFC) and nucleus reuniens (Re) of the thalamus, three regions necessary for successful working memory performance in rodents, in *Setd1a*-deficient mice. Adult male and female *Setd1a*$^{+/-}$ and C57/BJ wildtype (WT) littermate controls were trained in a delayed non-match-to-sample test of spatial working memory. Local field potentials were recorded in the mPFC, Re, and the CA1 region of the vHPC before and throughout task training. Experimenters were blinded to genotype. We observed that *Setd1a*$^{+/-}$ mice acquired the working memory task comparably to WT controls (p=0.633, WT, n=28; *Setd1a*$^{+/-}$, n=24, two-way ANOVA). We examined coherence, a measure of neural synchrony, prior to working memory training. *Setd1a*$^{+/-}$ mice displayed significantly lower mPFC-Re theta (4-12 Hz, p=0.02, two-way ANOVA) synchrony and non-significant decreases in gamma (30-80 Hz, p=0.09) synchrony relative to controls (WT, n=14; *Setd1a*$^{+/-}$, n=8). Conversely, among the vHPC-mPFC and vHPC-Re circuits, we observed trends toward an increase in gamma (vHPC-mPFC, p=0.27; vHPC-Re, p=0.08) and theta (vHPC-mPFC, p=0.15; vHPC-Re, p=0.21) synchrony before training. During task training, *Setd1a*$^{+/-}$ mice (WT, n=12; *Setd1a*$^{+/-}$, n=9) exhibited significant decreases in mPFC-Re theta

neural synchrony (p=0.001) while displaying no significant changes in gamma synchrony (p=0.15) compared to WT mice, similar to that seen before task learning. Moreover, we found no differences in vHPC-mPFC (WT, n=11; *Setd1a*$^{+/-}$, n=11) and vHPC-Re (WT, n=8; *Setd1a*$^{+/}$, n=11) theta (vHPC-mPFC, p=0.29; vHPC-Re, p=0.6) and gamma coherence (vHPC-mPFC, p=0.058; vHPC-Re, p=0.06) between *Setd1a*$^{+/-}$ mice and WT controls. These data suggest that alterations in synchrony among *Setd1a*$^{+/-}$ mice reflect stable alterations in connectivity within the mPFC-Re circuitry that exist prior to task training. Future studies aimed at validating these results will examine how vHPC and Re theta and gamma oscillations modulate mPFC neuronal spiking. Finally, understanding the neurophysiological consequences of haploinsufficiency of a definitive schizophrenia risk gene will provide insights into the neural mechanisms underlying the cognitive processes that are altered in this disorder.

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Poster

P059. Animal Models of Developmental Disorders

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Program #/Poster #: P059.04

Topic: H.13. Schizophrenia

Support: FRAXA Postdoctoral fellowship
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NIH Grant U54HD090256

Title: Schizophrenia risk gene FXR1 maintains prefrontal parvalbumin interneuron functions through enhancing Cav3.2 expression

Authors: *M. SHEN[1], Y. GUO[1], Q. DONG[2], Q. CHANG[4], X. ZHAO[3];
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Abstract: FXR1, a mutation-intolerant and high confident risk gene for schizophrenia, encodes a brain-enriched RNA binding protein, but its function in the brain remains unclear. Parvalbumin interneurons (PVI) are essential for sensory processing, working memory, and cognition and are impaired in psychiatric disorders including schizophrenia. We show that FXR1 has important roles in PVIs. Deletion of FXR1 from PVIs leads to reduced PVI excitability and synaptic transmission, impaired prefrontal cortical gamma oscillation and synchronization, and behavioral deficits resembling psychiatric disorders. PVI-specific translational profiling reveals that FXR1 binds Cacna1h encoding a T-type calcium channel Cav3.2. Inhibition of Cav3.2 phenocopies

FXR1 deficiency whereas elevation of Cav3.2 in PVIs or stimulation of PVI activities rescues behavioral deficits of mice with FXR1 deficiency. We have unveiled the function of a newly identified schizophrenia risk gene FXR1 in PVI excitability, circuit function, and behavior. We have identified Cav3.2 as a potential target for treating psychiatric disorders.

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Poster

P059. Animal Models of Developmental Disorders

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Support: NIH P50 Grant MH103222

Title: Longitudinal assessment of developmental changes in the structure and function of white matter tracts in adolescent minipigs

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Abstract: Most translational animal models used to study structural and functional alterations in the organization of cerebral white matter in schizophrenia have serious limitations. Rodents have a lissencephalic cortex and a grey:white matter ratio that differs from humans; ferrets undergo gyrification after birth; and use of non-human primates is constrained by ethical and economic considerations. By contrast, Sinclair miniature pigs are born with a fully gyrencephalic brain and have a grey:white matter volume ratio similar to humans. Pigs mature over a 4-6 month interval which is more extensive than rodents but compressed relative to primates, making them well suited for longitudinal studies of adolescent brain development. In the present series of experiments, we used quantitative diffusion weighted imaging (DWI) and evoked potential (EP) recording to assess the maturation of cerebral white matter in adolescent Sinclair miniature pigs. Animals underwent four EEG/MRI sessions on PND 98, 138, 166 and 194. Imaging was conducted in a Siemens 3 Tesla Prisma scanner and a 15-channel knee coil. A higher resolution anatomical (0.5mm isotropic) DWI protocol (1.25mm isotropic), proportionally scaled to the resolution used in humans, consisted of 15 b-shells (b = 0-3500 s/mm2) with 32-directions/shell. Data were analyzed using diffusion kurtosis and bi-exponential modeling that provide fractional anisotropy radial kurtosis, kurtosis anisotropy, axial kurtosis, tortuosity, and permeability-diffusivity index of cerebral white matter. EEG recordings were acquired with a Neuroscan

Synamp2 (5 kHz sampling rate; bandpass of 0.1-200 Hz) immediately before imaging from subdermal platinum-iridium needles. Following a 5-minute resting EEG recording, monocular visual EPs were elicited by light flashes (300 stimuli @ 1 Hz, 10 - 500 ms duration) generated by a Lifelines photic stimulator. Auditory EPs were recorded in duration mismatch (80% standard, 60-ms 1000 Hz tones and 20% deviant 100 ms, tones presented in pseudorandom order) and auditory steady-state (75 click trains presented at 2.5 Hz and 40 Hz) procedures. All stimuli were applied at 72 dB through inserted earphones (Etymotic ER-1). We expect to observe significant changes in DWI parameters consistent with the structural maturation of cerebral white matter during adolescence. We further anticipate that these changes will be reflected by coincident changes in the amplitude and conduction speed of visual and auditory EPs. These studies provide foundational experiments for assessing the role of elevated kynurenic acid in white matter abnormalities and related cognitive impairments in people with schizophrenia.

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Poster

P059. Animal Models of Developmental Disorders

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Program #/Poster #: P059.06

Topic: H.13. Schizophrenia

Support: R01MH104450
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T32-AA007468

Title: Dopamine neuron-specific manipulation of GRIN2A or GRIN2B influences adolescent behavior and psychostimulant sensitivity

Authors: M. L. KIELHOLD[1], D. S. JACOBS[1], A. P. BOGACHUK[1], A. HUNKER[2], L. ZWEIFEL[3], B. MOGHADDAM[1];
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Abstract: Background: NMDA receptor dysfunction has long been implicated in the etiology of schizophrenia and other neurodevelopmental disorders. Recent whole exome/genome sequencing studies have further associated variants in genes encoding the GluN2A and GluN2B subunits of the NMDA receptor (GRIN2A and GRIN2B respectively) with schizophrenia diagnosis.

Critically, levels of these subunits continue to be dynamically regulated through adolescence and early adulthood, when symptoms of schizophrenia manifest. We are interested in studying the effect of cell-specific disruption of GluN2A or GluN2B during adolescence on behaviors and related functions that are relevant to symptoms of schizophrenia. Our focus here is on levels of GluN2A and GluN2B in dopamine neurons within the VTA as these neurons have been historically implicated in the pathophysiology of schizophrenia.
Methods: Cre-dependent CRISPR/SaCas9 viruses for GRIN2A and GRIN2B were injected into the VTA of th::cre male and female rats after weaning. Adolescent animals were tested on a battery of behavioral tasks and for sensitivity to psychostimulants. Postmortem tissue was analyzed by IHC or western blotting for GluN2A or GluN2B abundance.
Results: Treatment with virus led to a partial reduction of GluN2A (40%) and GluN2B (60%) in the VTA. Knockout (KO) of either GRIN2A or 2B in dopamine neurons of adolescent male or female rats did not influence baseline motor behavior in the open field or simple associative learning. Differences began to emerge in more complex paradigms such as the progressive ratio schedule of reinforcement, where response rates were disrupted in different ways for GRIN2A and GRIN2B KO groups suggesting that these manipulations cause hyperactivity and changes in incentive value of reward, respectively. In addition, we observed sex-specific effects of propsychotic drugs with amphetamine producing an exaggerated response in GRIN2B KO males and MK801 producing an exaggerated response selectively in GRIN2A KO females.
Conclusion: While further characterization is necessary, our results suggest that selective KO of GRIN2A and GRIN2B in adolescent dopamine neurons may provide useful models for some aspects of genetic vulnerability to schizophrenia.

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Poster

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Topic: H.13. Schizophrenia

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Title: Evidence for a potential role of an increased copy number of the gene encoding glycine decarboxylase (GLDC) in the pathophysiology of psychosis

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Abstract: Genomic copy number variants (CNVs) have been implicated in the etiology of schizophrenia (SCZ) and bipolar disorder. In two patients with psychosis, a rare 9p24.1 CNV, a 1.8 Mb duplication/triplication involving multiple genes, including the GLDC gene encoding the glycine-degrading enzyme glycine decarboxylase, has been found by others. Our goal was to determine whether this CNV is sufficient to induce biochemical or behavioral phenotypes in mice and which gene(s) would be underlying these phenotypes. We hypothesized that an increased copy number of Gldc would lead to increased degradation of glycine, eventually resulting in NMDA receptor hypofunction and schizophrenia-like phenotypes. To address this, we developed mouse models with a triplication (4 copies) of the 9p24.1 genes, or a duplication (3 copies) or a triplication (4 copies) of Gldc alone or of all other 9p24.1 genes. These mice were subjected to qRT-PCR (n=5 each genotype), Western blot (n=10 each genotype) and behavioral studies (n=10 each genotype) at 2-4-month of age. In all genetic mouse models with increased copy numbers of Gldc we found an increase in GLDC protein in hippocampus (Hip), prefrontal cortex (PFC) and amygdala. The microRNA mir132 which has been linked to density of dendritic spines and which is downregulated in patients with schizophrenia was reduced in Hip and increased in PFC. In addition, BDNF mRNA was reduced in Hip and PFC of mice with 4 Gldc copies. However, expression of CREB, p-CREB and BDNF proteins was unaltered. MicroRNA mir137, the product of a risk gene for SCZ as evidenced by GWAS studies was increased in PFC. Furthermore, only in mice with increased Gldc copy number but not in mice with increased copy numbers of the other 9p24.1 genes, we found key behavioral deficits, i.e., startle habituation impairment, absence of latent inhibition to conditioned freezing, working memory deficits in both the Y- maze spontaneous alternation test and T- maze forced alternation tests and sociability deficits, along with social novelty preference deficits. Thus, an increase in the copy number of the Gldc gene is sufficient to induce molecular and behavioral features consistent with a schizophrenia-like phenotype. Our results suggest that in the patients with the 9p24.1 duplication/triplication the increase in GLDC copy number may be an important contributing factor to pathophysiology. The results are in line with our still to be tested overall hypothesis that an increased copy number of Gldc results in increased glycine degradation in astrocytes, leading to deficits in glycine-dependent activation of excitatory glutamate receptors of the NMDA type in neurons and NMDA receptor hypofunction.

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Poster

P059. Animal Models of Developmental Disorders

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Program #/Poster #: P059.08

Topic: H.13. Schizophrenia

Support: NIH Grant MH-058846
Emory Department of Psychology

Title: Vulnerability of parvalbumin circuits in basolateral amygdala following neonatal hippocampal damage in a monkey model of neurodevelopmental disorders

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Abstract: Schizophrenia (SCZ) and related neurodevelopmental disorders are associated with alterations in circuits involving the hippocampus, amygdala, and prefrontal cortex. In particular, early damage to the hippocampus may contribute to the pathophysiology of SCZ by inducing maldevelopment of GABAergic circuits within the fronto-limbic system. In monkeys, damage to the hippocampus early in life produces alterations in socioemotional behavior, underscoring a possible contribution of shifts in maturation of the amygdala. Finally, parvalbumin (PV) is a calcium-binding protein characteristic of subpopulations of GABA neurons that have been implicated in SCZ. Thus, we predicted that a neonatal hippocampal lesion would disrupt the maturation of PV cell populations within the amygdala. We used tissue from adult rhesus monkeys (*M. mulatta*) that had sustained bilateral ibotenic acid lesions of the hippocampus as neonates (Neo-H, n = 5) or sham controls (n= 6) to analyze the morphologies of PV+ interneurons and relative density of PV+ neuropil in the basal amygdala. PV cells were classified based on the descriptions of Pitkanen & Amaral (1993) in which Type I ('simple' in our terminology) neurons are mainly small and round, whereas Types II and III have more elaborate morphologies. We classified all PV+ neurons at three levels within the dorsal and ventral portions of the basal nucleus and the paralaminar nucleus to obtain a ratio of neurons with simple vs. elaborate morphologies. PV+ fibers were counted using a stereological strategy and expressed as a ratio of mean fiber density in DB:VB. Amygdalae from Neo-H cases had lower ratios of simple: elaborate neurons in both subdivisions of the basal nucleus, suggesting that early hippocampal lesions interfere with normal maturational processes of inhibitory cell morphology in the primate amygdala. Amygdalae from Neo-H cases also showed a strikingly lower density of PV+ fibers in the dorsal basal relative to the ventral basal in comparison with the equivalent ratio in controls, suggesting a redistribution of PV+, GABAergic connections from the dorsal to the ventral portion of the basal nucleus. Together, the results indicate that early lesions of the hippocampus in monkeys produce significant alterations in the internal inhibitory circuitry of the amygdala, and highlight the vulnerability of this PV+ population to

early-life perturbation. These results indicate that the effects of neonatal hippocampal lesions on behavior and frontal lobe function may include disruptions of the development of specific amygdala circuitry as part of a broader profile of limbic-frontal disconnection in neurodevelopmental disorders.

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Poster

P059. Animal Models of Developmental Disorders

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Topic: H.13. Schizophrenia

Support: Natural Sciences and Engineering Research Council of Canada (NSERC) – Alexander Graham Bell Canada Graduate Scholarship
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Title: Perinatal omega-3 fatty acid supplementation rescues the aberrant cognitive and affective symptomology in a prenatal cannabis exposure model of schizophrenia

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Abstract: Clinical and preclinical studies indicate prenatal cannabis exposure (PCE) pathologically effects fetal brain development and may increase vulnerability to neuropsychiatric disorders, including schizophrenia, cognitive and mood/anxiety disorders. However, the underlying mechanisms remain unknown. Research in our lab suggests that fetal exposure to Δ9-tetrahydrocannabinol (THC) impairs neurodevelopment, in part, through the alteration of cortical fatty acids. Considerable evidence demonstrates that abnormal omega-3 (N3) fatty acid signaling may underlie various neuropsychiatric disorders. In addition, evidence has shown that dietary interventions with N3 fatty acids may prevent or ameliorate neuropsychiatric symptom profiles. The present study examined if perinatal supplementation with N3-fatty acids may rescue neuronal, behavioural and molecular neuropsychiatric phenotypes induced by pre-natal THC exposure. Pregnant Wistar rats were assigned to saline (VEH) or 3mg/kg THC (daily, *i.p.*) from gestational day (GD) 7-22. Dams were given an N3 enriched diet vs. standard control diet (control: CT) *ad libitum* from GD 7 to postnatal day (PND) 21 of the offspring. Treatment groups were: VEH/CT, VEH/N3, THC/CT and THC/N3. On PND 21, a subset of male and

female offspring was sacrificed for molecular analyses. Between PND 35-45, and PND 90-120, subsets of male/female offspring underwent behavioural tests for memory, social and novelty-induced anxiety, sensory integration, and depression. The subsets were later used for *in vivo* electrophysiology or molecular analyses and Matrix-Assisted Laser Desorption/Ionization Imaging Mass Spectrometry. Our adolescent behavioural data indicated a sex-specific N3-mediated therapeutic effect on PCE-induced behavioural phenotypes. For the male rats, VEH/N3s exhibited significantly less anxiety than either the VEH/CT or THC/CT groups, but the THC/N3 group showed a similar level of anxiety as the THC/CT group. However, the N3 diet prevented memory and sociability impairments seen in the male THC/CT group. The female VEH/N3 and THC/N3 groups exhibited significantly lower anxiety and improved social and working memory compared to their THC/CT group. Thus, female rats experience a broader therapeutic effect than males. Given that N3's interact with estrogen receptors, this interaction will be further explored. Behavioral, electrophysiological, molecular, and fatty acid profile analyses are currently ongoing. These findings suggest that dietary interventions aimed at N3-fatty acid normalization may be a promising therapeutic option for cannabis-induced neurodevelopmental pathologies.

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Poster

P059. Animal Models of Developmental Disorders

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Program #/Poster #: P059.10

Topic: H.13. Schizophrenia

Support: NIH F31 MH119691

Title: Adolescent thalamic inhibition leads to long-lasting impairments in prefrontal cortex function

Authors: *L. J. BENOIT[1], E. HOLT[1], L. POSANI[1], S. FUSI[2], A. HARRIS[1], S. E. CANETTA[3], C. KELLENDONK[4];
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Abstract: Impaired cortical maturation is postulated as a mechanism in the etiology of neurodevelopmental disorders, including schizophrenia. In sensory cortex, activity relayed by the thalamus during a sensitive postnatal time window is essential for proper cortical maturation. Whether thalamic activity also shapes prefrontal cortical maturation is unknown. Here, we show

that inhibiting the midline thalamus during adolescence leads to a long-lasting decrease in the density of thalamo-prefrontal projections and attenuated cortical excitation. Thalamic inhibition during adolescence also causes prefrontal-dependent cognitive deficits during adulthood that are associated with disrupted single unit cross-correlations and impaired task outcome encoding in the prefrontal cortex. In contrast, inhibiting the thalamus during a comparable time window in adulthood has no long-lasting consequences. Strikingly, exciting the thalamus in adulthood during a cognitive task rescues prefrontal cross-correlations, task outcome encoding, and the cognitive deficit. These data point to adolescence as a sensitive time window of thalamo-cortical circuit maturation. Furthermore, by supporting prefrontal network activity and encoding, boosting thalamic activity provides a potential therapeutic strategy for rescuing cognitive deficits in neurodevelopmental disorders.

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Poster

P160. Microglia: Biology I

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Program #/Poster #: P160.01

Topic: B.11. Glial Mechanisms

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Title: Mechanisms of cerebellar microglia dynamics

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Abstract: Synaptic plasticity allows the central nervous system (CNS) to incorporate new sensory experiences and information, and its disruption is associated with many neurological and psychiatric disorders. Much recent work has focused on the contribution of non-neuronal CNS cells, especially microglia, to synaptic plasticity. Though classically thought of in their immune capacities, microglia are vital to many homeostatic processes, including synaptic plasticity of nascent and adult neuronal networks. Despite the emerging consensus that microglial dynamics are critical to brain function during physiological as well as pathological conditions, it is unclear whether these microglial roles and their underlying mechanisms are universal or differ between

brain regions. There is a growing body evidence to suggest microglia exhibit a high degree of regional specialization; existing on a continuum from homeostatic (cortex) to immune vigilant (cerebellum) even in the absence of pathological stimuli. Indeed, microglia in the cerebellum represent a distinct population, exhibiting unique transcriptional and epigenetic profiles, along with distinct functional properties, such as being more phagocytic, morphologically less ramified and less densely distributed. As a consequence, cerebellar microglia survey less of parenchyma than cortical microglia, but compensate for this by undergoing frequent somatic translocations under homeostatic conditions, a phenomenon not observed in cortex. Despite these differences, cerebellar microglia maintain common microglial functions, exhibiting a robust injury response and dynamic interactions with surrounding neural elements. Two pathways of interest to cortical microglial mediated synaptic plasticity include noradrenergic signaling through the β_2 adrenergic receptor (β_2-AR) and purinergic signaling through the P2Y12 receptor, both of which have been shown to be critically involved in microglial roles in synaptic remodeling and rapid chemotaxis to sites of injury. We used time-lapse *in vivo* imaging through a chronic cranial window preparation to visualize cerebellar microglial dynamics while β_2-AR and P2Y12 signaling were manipulated. We found that β_2-AR stimulation reduced microglial surveillance in both the cortex can cerebellum but slightly reduced cerebellar soma migration. Lastly, we demonstrated that P2Y12 deficiency did not alter the response of cerebellar microglia to focal injury, unlike what is seen in cortical microglia. These findings show that cerebellar microglia likely use a distinct set of molecular cues to guide dynamic responses in both homeostatic and pathological conditions.

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Poster

P160. Microglia: Biology I

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Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P160.02

Topic: B.11. Glial Mechanisms

Support: Conte Grant 71171
HHMI Grant CC34830

Title: Dissecting the regulation of a spatiotemporally distinct microglial state in postnatal development

Authors: *Y. DE SOYSA[1,3,4], A. C. WALKER[1], A. J. WALKER[1,3,4], B. STEVENS[1,3,4,2];
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Abstract: Microglia are brain-resident macrophages that regulate critical processes in neurodevelopment; dysregulation of microglial identity has been linked to neurodevelopmental disorders. Single-cell transcriptomics studies have identified dynamic microglial states throughout life, with the greatest heterogeneity appearing during the embryonic and early postnatal period. These states express a core gene signature defining their microglial identity, but also exhibit unique transcriptional programs indicative of discrete behaviors and functions. A critical gap in knowledge centers on how these states are transcriptionally regulated in distinct developmental contexts. Precisely mapping the mechanisms of microglial state regulation during normal development provides a framework for understanding how their perturbation contributes to neurodevelopmental disease progression and informing the development of therapeutic strategies. Axon Tract-associated Microglia (ATM) are a highly dynamic state with a distinct transcriptional profile that appears in the white matter axon tracts of the corpus callosum and cerebellum during the first postnatal week in mice, and in late gestation in humans. Due to their distinct gene signature and defined spatiotemporal window of emergence, ATM represent a powerful *in vivo* paradigm to interrogate how the intersection of developmental origin, intrinsic gene regulatory programs and environmental signals shapes the transcriptional signature of a microglial state. In this work, we combined ATAC-sequencing with ATM-specific cre ablation and fate-mapping reporter models to dissect how ATM emergence and attrition are regulated. First, we identified candidate transcription factors that drive and distinguish the ATM gene signature from that of canonical microglia at P5, the peak of their emergence, with ATAC-seq analysis. Second, developmental ablation of these cells revealed that a compensatory population with a distinct chromatin accessibility profile upregulated the ATM signature to repopulate the compartment. Finally, fate mapping analysis revealed that ATM attrition does not occur by apoptosis, but that ATM downregulate their defining gene signature, become reintegrated into the broader homeostatic microglia pool and persist in the white matter into adulthood. Capture of ATM chromatin profiles during this transition revealed putative signaling and transcriptional drivers underlying this dynamic reintegration process. Overall, this work uncovers novel insights into the transcriptional dynamics and context-specific regulatory mechanisms of a distinct *in vivo* microglial state.

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Poster

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Topic: B.11. Glial Mechanisms

Support: NIH R01HL142752
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Title: Pharmacologic microglial depletion disrupts breathing and body temperature regulation during torpor in 13-lined ground squirrels

Authors: R. J. SPRENGER[1], A. B. RADCLIFF[2], A. L. MEZA[2], C. R. MICKELSON[2], A. WATTERS[2], W. MILSOM[1], H. V. CAREY[2], *J. WATTERS[2], T. BAKER[2];
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Abstract: Mammalian hibernation initiates dramatic changes in physiology that include large reductions in ventilation ($\dot{V}_E$), heart rate, oxygen consumption rate ($\dot{V}o_2$), and body temperature (T_b) upon entrance into torpor, effects that reverse upon arousal. Several physiologic processes must maintain function throughout a wide range of temperatures. Recent evidence indicates that neuronal synapses loosen and retract in regions of the brain that are silenced during a torpor bout, although little is understood yet about how these connections are regulated during hibernation. Microglia, brain resident immune cells, are potential facilitators of these essential neural homeostatic activities due to their critical roles in neuronal support and synaptic strengthening and pruning. Thus, we hypothesized that microglia are required for maintaining normal ventilatory neural control during hibernation. To test this, we treated 13-lined ground squirrels with either vehicle or colony-stimulating factor receptor-1 (CSF1R) antagonist, PLX3397 (80mg/kg, po), a receptor whose activation is necessary for microglial survival in the adult nervous system, and measured $\dot{V}_E$, $\dot{V}o_2$, and T_b throughout a torpor bout. During entrance, PLX-treated squirrels took ~1.5 hours longer to reach their minimum torpor temperature (T_b; 12°C) compared to control animals (T_b; 9.3°C) (ambient temperature; 5-6°C). Similarly, arousal time in microglia-depleted animals was as much as 1 hour longer relative to controls, despite starting at a higher minimum T_b. In both treatment groups, $\dot{V}o_2$ during entrance fell and spiked periodically. However, in PLX-treated animals, these spikes were much greater and more frequent. Minimum $\dot{V}o_2$ in torpor was ~3 times greater in PLX-treated animals compared to control animals. Upon arousal from torpor, $\dot{V}o_2$ remained elevated, longer, in PLX-treated squirrels. Total $\dot{V}_E$ tended to be elevated in PLX-treated animals through entrance, steady state torpor, and arousal, but it was most striking during steady state torpor, where $\dot{V}_E$ in PLX-treated animals was as high as 15-fold greater than control animals. The net result in torpor was an elevated air convection requirement, thus microglia-depleted animals hyperventilated throughout the torpor bout. Together, these results suggest that microglia play an important regulatory role in normal ventilatory and temperature control throughout torpor and influence the rate at which animals enter into and arouse from torpor. Mechanisms whereby microglia contribute to hibernation neurophysiology are currently under investigation. (Funded by NIH R01HL142752 (JJW and TLB), NSF IOS1558044 (HVC), and NSERC 22R87150 (WKM)).

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Poster

P160. Microglia: Biology I

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Topic: B.11. Glial Mechanisms

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Title: Microglial phagocytosis modulates cellular metabolism and function, and remodels the mitochondrial network

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Abstract: Microglia are the immune cells of the brain and expert phagocytes of harmful content for parenchymal homeostasis. Apoptotic neurons generated during development, in adult neurogenic niches, and neurodegenerative conditions leak toxic intracellular content compromising surrounding cells. Microglial phagocytosis avoids the spillover of toxic content and exerts immunomodulatory effects. However, we still do not know the molecular, metabolic, and functional effects of engulfing and degrading an apoptotic cell. To address this question, we first performed a global transcriptomic analysis of primary microglia exposed to apoptotic cells. The analysis showed that phagocytosis induced changes in metabolic gene expression, suggesting a positive regulation of glycolytic genes and a negative regulation of oxidative phosphorylation genes. We used Seahorse extracellular analyzer to confirm the reduction of mitochondrial metabolism, although we did not detect increased glycolysis. Phagocytosis-

induced metabolic changes were related to a strong remodeling of the mitochondrial network, with fewer and less complex mitochondria. These metabolic and mitochondrial changes compromised cell function, leading to a reduced engulfing capacity of microglia 24 hours after phagocytosis. Furthermore, to study phagocytosis-induced changes in vivo, we developed a "superphagocytosis" model using low cranial irradiation (2Gy), which induced apoptosis of newborn cells in the dentate gyrus and subsequent increase in microglial phagocytosis. This model offers several advantages to study phagocytosis in vivo, as it considerably increases the number of phagocytic microglia without damaging them or disrupting the blood-brain barrier (BBB). We are currently examining the transcriptome, ultrastructure, and function of phagocytic microglia by performing single-cell RNA-sequencing, electron microscopy, and functional analysis. This data will provide us with more information about the complex process of phagocytosis, a necessary knowledge to use microglia as a therapeutic target in diseases where phagocytosis is impaired.

Disclosures: M. Márquez-Ropero: None. **J. Valero:** None. **S. Beccari:** None. **A. Márquez-Galera:** None. **L. Ayerra:** None. **A. Vilas-Zornoza:** None. **I. Díaz-Aparicio:** None. **V. Sánchez-Zafra:** None. **F. García-Moreno:** None. **J. Gómez-Arozamena:** None. **K. Blomgren:** None. **M. Aymerich:** None. **J. López-Atalaya:** None. **I. Casafont:** None. **A. Sierra:** None.

Poster

P160. Microglia: Biology I

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P160.05

Topic: B.11. Glial Mechanisms

Support: NIH Grant 5R01GM134104

Title: Differences in microglia and macrophage interaction with extracellular matrix environment

Authors: *S. PAULSON, M. STINSON, J. ROTTY;
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Abstract: Microglia are becoming the forefront of neurodisease research as more studies develop the link between chronic microglial inflammation and disease pathways. To better understand what potential role these cells play in disease progression, it is important to first determine their interactions with the central nervous system (CNS) extracellular matrix (ECM). The ECM microenvironment is complex, containing at least two different types of cues: ECM composition and ECM stiffness. Our overarching hypothesis is that these two components will shift the behavior of microglia. Understanding the underlying mechanisms will help us appreciate how these components of the microenvironment influence microglia function. This

understanding can then potentially be translated to human disease settings. Previous research in our lab has established that the ECM microenvironment alters the migrational velocity and morphology of immortalized bone marrow derived murine macrophages without the presence of an extracellular gradient cue. We wondered whether we would replicate these results with immortalized murine microglia (BV2) cells and set out to compare their responses to those already established in macrophages. Findings that support similarities between microglia and macrophages would demonstrate that response to different ECM components is conserved across cell-types, while a divergent response would reveal important differences that might explain underlying physiological responses. We observed a morphological difference in microglia plated on the two ECM proteins fibronectin and laminin, as well as differences in velocity and directional persistence. BV2s demonstrated higher velocities on laminin, proceeding in a balled-up morphological state; BV2s plated on fibronectin demonstrated slower speeds and a more spread-out morphological state. These changes were similar to those we observed in macrophages. In addition, we performed motility experiments on variable stiffness polyacrylamide gels to more closely replicate the soft environment of the CNS compared to the glass dishes used in initial motility tests. Polyacrylamide gels at softer densities closer to that of the brain displayed increased velocity and directional persistence over gels with stiffer densities closer to that of glass. Ongoing experiments in the lab will continue to assess ECM context effects on microglia after they are activated by inflammatory cues. Future studies will determine the mechanistic character of the microglia ECM discrimination and mechanosensing pathways, and their in vivo relevance.

Disclosures: S. Paulson: None. **M. Stinson:** None. **J. Rotty:** None.

Poster

P160. Microglia: Biology I

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Program #/Poster #: P160.06

Topic: B.11. Glial Mechanisms

Support: NIH NINDS R01NS089688
U01NS113279

Title: Effect of neuronal cell adhesion molecule L1 on microglia grown on nanoparticle modified surfaces

Authors: *N. KUSHWAH, K. WOEPPEL, X. TRACY CUI;
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Abstract: ***Background:*** Neural recording electrodes often suffer from low recording yield and stability, which have been partly associated with the inflammatory host tissue responses to neural

probe implantation. The neuronal cell adhesion molecule L1 has been used as biomimetic coating for neural electrodes and demonstrated significant benefit in reducing glial scar formation, promoting electrode-neuron integration, and improving recording performance. Coating probes with L1 on a nanoparticle (NP) base layer further increased these beneficial effects. Two photon microscopy studies revealed that the L1 coating inhibits microglial spreading on neural probes at 2- and 6-hours post-implantation suggesting a specific interaction between the surface bound protein and microglia. However, the exact mechanism of L1-microglia interaction has not been understood. In this study we cultured primary microglia cells on various L1 modified surfaces (with and without nanoparticle base layer) in an effort to illustrate the exact effects of L1 and nanoparticle on microglia activation. ***Method:*** Microglial cells were extracted from E-18 fetus and grown on smooth and NP coated surfaces with and without L1. After stimulating with phorbol myristate acetate (PMA), Dihydrorhodamine 123 (DHR) staining and cytochrome c assay were performed to characterize the production of superoxide. ELISA for inflammatory markers was also performed following stimulation with lipopolysaccharide (LPS), to study inflammation. ***Results:*** Results showed reduced superoxide production, decreased pro-inflammatory cytokines (TNF alpha, IL-6, IL-1 beta) and increased level of anti-inflammatory markers (TGF beta 1 and IL-10) in L1 treated samples. Furthermore, microglia phagocytosis was studied and L1 significantly increased the number of phagocytic cells, which may translate to enhanced ability for removing the cellular debris caused by implantation injury. ***Conclusion:*** From these observations it can be concluded that L1 protein significantly reduces superoxide production and inflammatory response of microglial cells in response to insults and promote wound healing.

Disclosures: N. Kushwah: None. **K. Woeppel:** None. **X. Tracy Cui:** None.

Poster

P160. Microglia: Biology I

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Program #/Poster #: P160.07

Topic: B.11. Glial Mechanisms

Title: Microglial culture shift: Finding factors to drive in vitro microglia towards an in vivo phenotype

Authors: *R. MISSALL, F. GIGASE, R. KUBLER, G. SNIJDERS, E. J. GARCIA REINO, L. DE WITTE;
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Abstract: Primary adult microglia rapidly lose their microglial-specific phenotype when they are isolated from the central nervous system (CNS) and put in culture. The sensitivity of microglia to even small environmental changes creates difficulty in extending *in vitro* results into *in vivo*

hypotheses and makes comparisons between experimental outcomes inconclusive and even contradictory. These problems are exacerbated by the lack of a standardized microglia culture protocol accurately representing the *in vivo* CNS environment. To address this issue, we first define a multi-faceted target phenotype, which we term the mature homeostatic microglial (MHM) phenotype. This includes a radially ramified morphology, motile processes with low overall cell mobility, and an RNAseq-based human microglial gene signature. Notably, this gene signature makes up a subset of genes in previously published microglia-specific gene signatures, and includes commonly seen genes such as *p2ry12*, *cx3cr1*, and complement factors, as well as genes such as *folr2* and *insr*. We next performed a consensus weighted gene correlation network analysis (WGCNA) on RNAseq data of 410 human microglia samples from one published dataset and two of our own unpublished, a significant improvement on the strength of *ex vivo* vs. *in vitro* comparisons done to date. Key gene networks identified include those enriched for GO terms associated with myeloid differentiation and signaling, cell migration, and intracellular transport. Alongside WGCNA, we perform a structural literature review, and combine these results to build a list of factors that have been shown or are predicted to drive the MHM phenotype. This list includes factors already in use, such as CSF1 and IL-34, along with lesser investigated factors such as cholesterol and semaphorins. Lastly, we perform experiments to assess the effects of the identified factors on human primary microglia *in vitro*. Together, this will contribute to development of a standardized culture protocol that mimics the *in vivo* CNS environment, thereby better enabling molecular and cellular studies of adult primary microglia.

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Poster

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Program #/Poster #: P160.08

Topic: B.11. Glial Mechanisms

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Title: Minimizing the ex vivo confounds of cell-isolation techniques on transcriptomic profiles of purified microglia

Authors: *S. R. OCAÑAS[1], K. D. PHAM[2], H. E. BLANKENSHIP[1], A. H. MACHALINSKI[3], A. J. CHUCAIR-ELLIOTT[2], W. M. FREEMAN[2];
[1]Univ. of Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK; [2]Genes and Human Dis., [3]Oklahoma Med. Res. Fndn., Oklahoma City, OK

Abstract: Modern molecular neuroscience studies require analysis of specific cellular populations derived from brain tissue samples to disambiguate cell-type specific events. This is particularly true in the analysis of minority glial populations in the brain, such as microglia, which may be obscured in whole tissue analyses. Microglia have central functions in development, aging, and neurodegeneration and are a current focus of neuroscience research. A long-standing concern for glial biologists using *in vivo* models is whether cell isolation from CNS tissue could introduce *ex vivo* artifacts in microglia, which respond quickly to changes in the environment. Mouse microglia were purified by magnetic-activated cell sorting (MACS), as well as cytometer- and cartridge-based fluorescence-activated cell sorting (FACS) approaches to compare and contrast performance. The Cx3cr1-NuTRAP mouse model was used here to provide an endogenous fluorescent microglial marker and a microglial-specific translatome profile as a baseline comparison lacking cell isolation artifacts. All methods performed similarly for microglial purity with main differences being in cell yield and time of isolation. *Ex vivo* activation signatures occurred principally during the initial tissue dissociation and cell preparation and not the microglial cell sorting. Utilizing transcriptional and translational inhibitors during the cell preparation prevented the activational phenotype. These data demonstrate that a variety of microglial isolation approaches can be used, depending on experimental needs, and that inhibitor cocktails are effective at reducing cell preparation artifacts.

Disclosures: S.R. Ocañas: None. **K.D. Pham:** None. **H.E. Blankenship:** None. **A.H. Machalinski:** None. **A.J. Chucair-Elliott:** None. **W.M. Freeman:** None.

Poster

P194. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches III

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P194.01

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

Support: U54AG054349

Title: Identification and characterization of UCI MODEL-AD mouse models using single-cell and single-nucleus Split-seq

Authors: *H. LIANG, M. GANTUZ, N. REZAIE, G. BALDERRAMA-GUTIERREZ, C. MODEL-AD, A. MORTAZAVI;
Univ. of California, Irvine, Irvine, CA

Abstract: Identification and characterization of UCI MODEL-AD mouse models using single-cell and single-nucleus Split-seq
Authors: Heidi Y. Liang, Magdalena Gantuz, Narges Rezaie, Gabriela Balderrama-Gutierrez, MODEL-AD Consortium**,** Ali Mortazavi
Alzheimer's Disease (AD) is a progressive neurodegenerative disease that affects over 5 million Americans and is the 6th leading cause of death in the US. Scientists have been using the mouse brain as a pre-clinical model of human brain, but no existing mouse models of AD are able to recapitulate fully human AD pathology. The MODEL-AD consortium seeks to generate more realistic mouse models of AD using human GWAS variants to further understand the disease as well as to develop more effective therapeutics.
One of the core activities of MODEL-AD is to characterize the mouse models using transcriptomics, primarily using bulk RNA-seq. Here, we investigated the performance of the Split-seq (split-pool ligation-based transcriptome sequencing) protocol for single-cell and single-nucleus RNA-seq in mouse hippocampus and cortex. As a proof of concept, we compared 5xFAD, which shows heavy plaque load, and 3xTgAD, which have both plaques and tangles, using Split-seq. Specifically, we capture microglial cells from right half of the brain tissues, and isolate nuclei from the left half of the same brain, then profile the combined samples using Split-seq in the same sequencing run.
We discuss our current experimental pipelines and preliminary results which show that we can distinguish populations of activated microglia as well as distinct neuronal populations in the two mouse models. This suggests that Split-seq will be a useful platform of the late-onset models that MODEL-AD is in the process of releasing.

Disclosures: H. Liang: None. **M. Gantuz:** None. **N. Rezaie:** None. **G. Balderrama-Gutierrez:** None. **C. Model-ad:** None. **A. Mortazavi:** None.

Poster

P194. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches III

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P194.02

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

Support: NIA Grant U54 AG054345-01
NIH Grant P30 AG0380770

Title: Metabolomics Profiling Reveals Distinct Signatures in the Serum and Brain Metabolites in Mouse Models of Alzheimer's Disease

Authors: *R. PANDEY[1], M. ARNOLD[2,3], R. KADDURAH-DAOUK[4], G. W. CARTER[1]; [1]The Jackson Lab., Bar Harbor, ME; [2]Helmholtz Zentrum München, German Res. Ctr. for Envrn. Health,, Inst. of Computat. Biology,, Neuherberg, Germany; [3]Dept. of Psychiatry and Behavioral Sci., Duke University,, Durham, NC; [4]Psychiatry, Duke Univ. Med. Ctr., Durham, NC

Abstract: Progress in development of efficacious therapies for Alzheimer's disease (AD) is halted limited understanding of underlying pathological mechanisms. Increasing evidence suggests that metabolic impairments prior to symptom development can contribute to disease mechanisms and subsequent dementia. Signals in conserved metabolomic pathways could provide a method to translate experimental findings in preclinical mouse models to humans. In this study, we systematically investigated the sex-stratified associations of serum and brain metabolites with sample-matched APOE4.Trem2^{R47H}and 5xFAD mouse models at six months of age. Metabolites were measured withthe targeted metabolomics platform AbsoluteIDQ®-p180 (Biocrates AG, Innsbruck, Austria). We next investigated whether blood and brain metabolites significantly associated with AD biomarkers in the ADNI cohort and the ROSMAP cohort, respectively. We identified a sex-specific increase of glycerophospholipids levels in male mice, while levels of sphingolipids were more abundant in females. Further, we identified that serum levels of glycerophospholipidswere reduced in APOE4.Trem2^{R47H}mice compared to C57BL/6J controls, while levels of these metabolites in the same animals were greater in both male and female brains of APOE4.Trem2^{R47H} and 5XFAD mice. Several of these findings were consistent with recent results from the ADNI cohort, which suggested a similar decrease in the same metabolites in serum of APOE4 carriers compared to non-carriers and indicates a serum-based effect of APOE genotype. Metabolomic signatures were notably different between brain and serum in mouse models. The 5xFAD mice exhibited stronger effects in the brain, whereas the APOE4.Trem2^{R47H} mouse showed more pronounced effects in serum with reversed effects in the brain. These findings are consistent with high levels of amyloid pathology in 5xFAD mouse brains and the modifications of serum biomarkers in APOE4.Trem2^{R47H} mice, and demonstrate an important mismatch between serum and brain metabolite abundances.

Disclosures: R. Pandey: None. **M. Arnold:** None. **R. Kaddurah-Daouk:** None. **G.W. Carter:** None.

Poster

P194. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches III

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P194.03

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

Support: U54AG054349

Title: Analyzing gene expression during neurodegeneration in a 3xTgAD mouse model at transcriptome level

Authors: *N. REZAIE, G. BALDERRAMA-GUTIERREZ, H. LIANG, C. MODEL-AD, A. MORTAZAVI;
Univ. of California, Irvine, Irvine, CA

Abstract: Clinical trials for potential Alzheimer disease (AD) drugs have a high failure rate since most drugs are successful in mouse models but are not translatable to humans. Mouse models fail to fully recapitulate the pathology, especially presenting plaques, and tangles at the same time.
The 3xTg mouse model contains three mutations associated with familial AD (APP Swedish, MAPT P301L, and PSEN1 M146V), and it is the only AD model that presents plaques and tangles. While the 3xTg shows changes in behavior, the transcriptomic response of the different subtypes of neurons and glia during neurodegeneration has not been comprehensively characterized.
As part of the MODEL-AD consortium phenotypic characterization of existing models of familial AD, we use bulk RNA-seq in the female hippocampus of 3xTg mice at different time points (4,12 and 18 months) to identify distinct gene modules associated with microglia and oligodendrocytes that increase with ageing and pathology. We further investigate the changes in cell populations using matching single-nucleus RNA-seq of the hippocampus and cortex of 3xTg (12, 18 and 24 months) and 5xFAD (18 months) mice to detect major changes in astrocytes and oligodendrocytes groups. We recover a common path of astrocyte activation with the 5xFAD mouse and find that 3xTg derived astrocytes seem to be at an earlier stage of activation. To investigate the activation of microglia in 3xTG, we also generated a single-cell RNA-seq dataset of microglial cells and found multiple subtypes, including a set of microglia with distinct transcription factor expression profiles that are associated with an early increase in CSF1 expression before the full onset of DAM gene expression. Finally, scATAC-seq reveals a set of chromatin accessible areas shared across multiple activation states found in the scRNA-seq that matches glial activation processes. Overall, differences between the main glial groups point to a slower activation process in the 3xTg model when compared to the 5xFAD. Our study contributes to identifying progressive transcriptional changes of glial cells in a model with plaques and tangles.

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Poster

P194. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches III

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P194.04

Topic:

Support: NIA U54 AG054345

Title: Improved mouse models of the Alzheimer's disease risk variant Trem2*R47H

Authors: *M. SASNER[1], G. MACGREGOR[2], A. OBLAK[3], D. GARCEAU[1], K. KOTREDES[1], C. PREUSS[1], A. UYAR[1], R. PANDEY[1], P. B.-C. LIN[3], A. P.-Y. TSAI[3], S. KAWAUCHI[2], G. BALDERRAMA-GUTIERREZ[2], J. ALCANTARA[2], J. NEUMANN[2], A. MORTAZAVI[2], K. N. GREEN[2], G. R. HOWELL[1], G. CARTER[1], A. J. TENNER[2], F. M. LAFERLA[2], B. T. LAMB[3]; [1]The Jackson Lab., Bar Harbor, ME; [2]Univ. California Irvine, Irvine, CA; [3]Stark Neurosciences Res. Inst., Indianapolis, IN

Abstract: TREM2 (triggering receptor expressed on myeloid cells 2) is expressed by microglia in the brain and plays a key role in modulating microglial activity. TREM2 expression is increased in AD and is associated with recruitment of microglia to amyloid plaques. The TREM2*R47H allele is one of the strongest genetic risk factors for developing late-onset Alzheimer's disease; patients exhibit both earlier onset of symptoms and rapid cognitive decline. We used CRISPR to engineer the R47H variant into the mouse *Trem2* locus; this model is available as JAX #27918. This, as well as two other CRISPR-generated *Trem2**R47H models, displays a splicing event seen in mouse, but not humans, that leads to a novel splice form with a deletion of 119bp at the 5' end of exon 2 (Xiang et al, *Molecular Neurodegeneration,* 2018). This leads to a decrease in ~50% of both transcript and protein levels in brain homogenate in the mouse, indicating that the model expresses mutant *Trem2*, but is also a hypomorph. To generate a more clinically relevant model, MODEL-AD has undertaken two complementary approaches. One approach has modified the existing Trem2*R47H mouse allele by using CRISPR to humanize the exon 2 splice site. This model, which we are calling Trem2*R47H^{HSS} for **h**umanized **s**plice **s**ite (JAX #33781), was analyzed by RNA-seq of brain homogenate. Relative to B6J controls, this new model expresses normal levels of each of the four transcripts. No truncated transcript was detected.As a second approach, we used a CRISPR-based strategy to engineer the R47H variant into the *Trem2* locus in B6J mice, accompanied by a different set of synonymous base changes. RNA-seq analysis of hippocampus and cortex indicates that transcript levels are similar to the wild-type *Trem2* allele, with no evidence of cryptic splicing products from the mutant allele. This model, named Trem2*R47H^{NSS} for **n**ormal **s**plice **s**ites is also available (JAX # 34036). We expect that these newly available models will facilitate research into the mechanisms and pathways related to *TREM2* genetic risk in late-onset AD.For more information see www.model-ad.org .

Disclosures: M. Sasner: None. **G. MacGregor:** None. **A. Oblak:** None. **D. Garceau:** None. **K. Kotredes:** None. **C. Preuss:** None. **A. Uyar:** None. **R. Pandey:** None. **P.B. Lin:** None. **A.P. Tsai:** None. **S. Kawauchi:** None. **G. Balderrama-Gutierrez:** None. **J. Alcantara:** None. **J. Neumann:** None. **A. Mortazavi:** None. **K.N. Green:** None. **G.R. Howell:** None. **G. Carter:** None. **A.J. Tenner:** None. **F.M. LaFerla:** None. **B.T. Lamb:** None.

Poster

P194. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches III

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P194.05

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

Support: NIA grant U54 AG054345
NIA grant U54 AG065181
NIA grant K01 AG054753

Title: Inpp5d regulates the amyloid pathology in alzheimer's disease

Authors: *P. B.-C. LIN[1], A. P.-Y. TSAI[1], K. NHO[2], B. T. LAMB[1], A. OBLAK[3];
[1]Stark Neurosciences Res. Inst., Indianapolis, IN; [2]Dept. of Radiology & Imaging Sci., Indiana Univ. Sch. of Med., Indianapolis, IN; [3]Stark Neurosci. Res. Inst., Indianapolis, IN

Abstract: Background Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline, robust microgliosis, neuroinflammation, and neuronal loss. Genome-wide association studies highlighted a prominent role for microglia in late-onset AD (LOAD). Specifically, inositol polyphosphate-5-phosphatase (*INPP5D*) is selectively expressed in brain microglia and one of its common intronic variants (rs35349669; OR=1.08, 95%CI=1.06-1.11) has been reported to be associated with increased risk of LOAD. INPP5D is linked to the triggering receptor expressed on myeloid cells 2 (TREM2) signaling, but little is known about the function of INPP5D in microglia and how INPP5D regulates TREM2-related AD pathogenesis. Therefore, we aim to understand the role of INPP5D in microglia and AD pathology. **Methods** We performed differential gene expression analysis to investigate INPP5D expression in LOAD and its association with plaque density using transcriptomic (RNA-Seq) data from the Accelerating Medicines Partnership for Alzheimer's Disease (AMP-AD) cohort. We also performed quantitative real-time PCR, immunoblotting, and immunofluorescence assays to assess INPP5D expression and microglial markers in the 5xFAD amyloid mouse model with INPP5D deficiency. Using the INPP5D knockout animal crossed with the 5xFAD, we performed behavioral assays and IHC staining to reveal how the INPP5D regulates amyloid pathology. **Results** *INPP5D* gene expression is upregulated in LOAD and positively correlated with the amyloid plaque density. *Inpp5d* expression increased along with increasing disease progression in 5xFAD mice, and is selectively expressed in plaque-associated microglia. Furthermore, reducing Inpp5d expression *in vivo* mitigates plaque burden in 5xFAD mice and further protected against behavioral deficits induced by amyloid pathology. **Conclusions** We have demonstrated that INPP5D expression increases throughout the AD progression and is predominantly in plaque-associated microglia. Importantly, inhibiting INPP5D expression reduces amyloid pathology and protects against amyloid-induced behavioral deficits, thus highlighting INPP5D as a potential therapeutic target.

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Poster

P194. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches III

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Program #/Poster #: P194.06

Topic:

Support: NIA/1RF1AG069425-01
NIH RF1AG051495

Title: Cx3cr1 deficiency aggravates amyloid driven neurodegeneration and cognitive decline in alzheimer's disease

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Abstract: Signaling via microglial Cx3CR1and its neuronal ligand (Cx3CL1) has been long associated with 'protective' activation of brain resident glia, as well as protection against neuronal excitotoxicity. Recently, Cx3CR1-V429I, a putative loss-of-function variant, previously associated with macular generation has been associated with worsened tau pathology, Braak staging and neurodegeneration in AD and ALS. Despite its identification as a key checkpoint regulator of microglial activation in Alzheimer’s disease, the overarching role of CX3CR1 signaling in modulating mechanisms of Aβ driven neurodegeneration is not well understood. In this study we investigate accumulation and phagocytosis of soluble and insoluble Aβ species, plaque associated microglial activation, synaptic dysregulation and neurodegeneration with disease progression in 5xFAD;*Cx3cr1*$^{+/+}$ and 5xFAD;*Cx3cr1*$^{-/-}$ mice. Lastly, we use Y-maze testing to analyze the effects of *Cx3cr1* deficiency on working memory in 5xFAD mice. Disease progression in 5xFAD;*Cx3cr1*$^{-/-}$ mice is characterized by increased deposition of filamentous plaques that display defective microglial plaque engagement, along with impaired microglial Aβ phagocytosis and lysosomal acidification *in-vivo*. Interestingly, *Cx3cr1* deficiency resulted in heighted accumulation of neurotoxic, oligomeric Aβ, which correlated with severe neuritic dystrophy, preferential loss of post-synaptic densities, exacerbated tau pathology, neuronal loss and cognitive impairment. Transcriptomic analyses using cortical lysates revealed that biological pathways regulating apoptotic signaling, TGFb signaling, ROS production and responses to ER

stress are differentially altered in 5xFAD;*Cx3cr1*$^{-/-}$ mice. Interestingly, microglia purified from 6 month-old 5xFAD;*Cx3cr1*-/- mice show increased mRNA levels of pro-inflammatory cytokines CCL2, CCL5 and IL1b while TNF and IL6 levels were significantly downregulated as compared to microglia from age-matched 5xFAD;*Cx3cr1*$^{+/+}$ mice. Lastly, Cx3cr1 deficient microglia show a significant downregulation of TGFb1 mRNA with elevated transcript levels of PTEN and Cybb. Thus, *Cx3cr1* deficiency impairs microglial uptake and degradation of fibrillar Aβ, thereby triggering an accumulation of neurotoxic Aβ species. Furthermore, loss of Cx3CR1 also results in a largely pro-inflammatory microglial activation phenotype, with a dysregulation in TGFb signaling, ER/oxidative stress and ROS metabolism. Our results indicate that Aβ-driven microglial dysfunction in *Cx3cr1*$^{-/-}$ mice aggravates tau hyperphosphorylation, neurodegeneration, synaptic dysregulation and impairs working memory.

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Poster

P194. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches III

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P194.07

Topic:

Support: NIH AG054345
NIH AG054753

Title: High Fat Diet Exacerbates the Neuroinflammatory Signature in Mouse Models relevant to Late-Onset Alzheimer’s Disease

Authors: *A. L. OBLAK[1], K. KOTREDES[2], R. PANDEY[2], M. SASNER[2], S. J. SUKOFF RIZZO[3], G. W. CARTER[2], B. T. LAMB[1], G. R. HOWELL[2];
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Abstract: High Fat Diet Exacerbates the Neuroinflammatory Signature in Mouse Models relevant to Late-Onset Alzheimer’s Disease
Adrian L. Oblak, Kevin Kotredes, Ravi Pandey, Michael Sasner, Stacey Rizzo, Greg Carter, Bruce Lamb, Gareth Howell
Age, genetic variants, and environmental factors such as consumption of a high fat diet contribute to Alzheimer’s disease (AD) risk. Diets high in fat and sugar also increase disease neuropathology and/or cognitive deficits in mouse models relevant to early-onset or familial AD. However, the effect of a high-fat diet on biometrics, neuropathology and cognitive impairments

in novel knock-in models of late-onset AD (LOAD) is unknown. The IU/JAX/PITT MODEL-AD Center is focused on developing improved mouse models for LOAD by combining genetic risk variants such as *APOE^{E4}*, *Trem2*R47H*, and *Plcg2*M28L* initially on a C57BL/6J (B6) background. Here, we examine the effect of incorporating a diet high in fat and sugar as an environmental risk factor to improve alignment of novel mouse models to LOAD. A high fat diet (45% high fat/high sugar, HFD) was provided *ad libitum* to B6.APOE^{E4}TREM2^{R47H} (LOAD1), and LOAD1.Plcg2^{M28L} mice from two to 12 months of age. Control mice from each strain were fed standard chow diet (6% fat). Metabolic assays, including weight and blood glucose, were performed throughout the study. Cholesterol, liver enzymes, cytokines, transcriptomics and AD-relevant neuropathology were performed at terminal timepoints. As expected, consumption of a HFD exacerbated age-related increases in body weight in both LOAD1 and LOAD1.*Plcg2^{M28L}* mice. Further, HFD increased glucose, cholesterol, triglycerides and liver enzymes regardless of genotype. Interestingly, HFD had different effects in the periphery compared to the CNS. In HFD LOAD animals, we found an increase in plasma proinflammatory cytokines TNFα and CXCL1, but reduced IL2 and IFNγ. In brain, IFNγ and IL2 are increased in LOAD1 and LOAD1.Plcg2^{M28L} animals. Transcriptomic analysis of brain samples revealed that the presence of the *Plcg2^{M28L}* risk variant in combination with HFD caused an elevation of human AD-relevant inflammatory genes that didn't occur in LOAD1 on HFD or in LOAD1.*Plcg2^{M28L}* mice on control diet. Our multi-analyte approach suggests that changes in inflammatory signature is exacerbated by the interaction of genetic risk factors for LOAD and HFD. These findings provide a platform to combine LOAD variants and environmental influences, providing more relevant models for preclinical studies.

Disclosures: A.L. Oblak: None. **K. Kotredes:** None. **R. Pandey:** None. **M. Sasner:** None. **S.J. Sukoff Rizzo:** None. **G.W. Carter:** None. **B.T. Lamb:** None. **G.R. Howell:** None.

Poster

P194. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches III

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P194.08

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

Support: NIMH MH107487
NIMH MH121102

Title: Analysis of CNS disorders using active kinome profiles

Authors: *A. JOYCE, K. ALGANEM, A. S. IMAMI, J. CREEDEN, A. HAMOUD, W. RYAN, R. SHUKLA, R. MCCULLUMSMITH;
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Abstract: Central nervous system (CNS) disorders such as AD (Alzheimer's dementia) and schizophrenia are often caused by a variety of pathophysiological processes, many of which involve abnormal protein kinase signaling. The active kinome is the set of kinases actively regulating cellular functions through phosphorylation. The active kinome can be profiled using protein kinase activity arrays. Using these profiles, we can empirically determine the phosphorylation state of peptide targets and find commonalities between the mechanisms of CNS disorders. To determine how the active kinome regulates the mechanisms underlying CNS disorders, we will first identify peptides that are differentially phosphorylated in AD and schizophrenia postmortem brain samples versus control subjects. We will then use deconvolution methods to identify the top dysregulated kinasesTo determine how the active kinome regulates the mechanisms underlying CNS disorders, we will first validate kinases that have been implicated in CNS disorders by our kinome array profile. We will identify peptides on the array that are differentially phosphorylated in Alzhemier's dementia and schizophrenia. Next, techniques such as BLAST will be used to assign these peptides to their respective proteins and identify homologous sequences mapped to other proteins. We will use perform an in-silico data exploration of databases such as BrainAtlas to determine the regional and cell level protein expression patterns of both the kinases and targets in the brain. We will use Enrichr to perform a pathway analysis on the final list of proteins to determine their functional implications.To have a more thorough understanding of the effects of the active kinome and the downstream effects of its protein targets, we will compare the two sets of pathways generated from ADAlzheimers and schizophrenia. Finally, we will curate publicly available CNS datasets from phosphoproteomic databases of CNS datasets disorders and explore the overlapping pathways using these data sets to get a more complete picture of the commonalities between these disorders regarding dysregulated kinases. We believe that this dysregulation plays an important role in CNS disorders and that common pathways regarding these dysregulated kinases could provide a unique insight into the mechanisms of CNS disorders and their methods of treatment.

Disclosures: A. Joyce: None. **K. Alganem:** None. **A.S. Imami:** None. **J. Creeden:** None. **A. Hamoud:** None. **W. Ryan:** None. **R. Shukla:** None. **R. McCullumsmith:** None.

Poster

P194. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches III

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P194.09

Topic:

Support: NIMH MH10748
NIMH MH121102

Title: Protein Kinase B Expression and Activity in Schizophrenia and Alzheimer's Dementia

Authors: *P. PULVENDER, N. D. HENKEL, E. A. DEVINE, R. E. MCCULLUMSMITH;
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Abstract: Protein Kinase B (AKT) Expression and Activity in Schizophrenia and Alzheimer's Dementia Authors: Priyanka Pulvender, Nicholas Henkel, Emily A. Devine and Robert McCullumsmith.Schizophrenia (SCZ) and Alzheimer's dementia (AD) are devastating illnesses that impact millionsworldwide. SCZ, a severe psychiatric disorder characterized by psychotic and delusional symptoms as wellas cognitive impairment, affects over 2 million in the United States and 50 million worldwide. AD, themost common cause of dementia, impacts over 5.7 million people in United Sates, and 50 millionworldwide.AKT, or Protein Kinase B, is a serine/threonine-specific protein kinase that plays a key role inmultiple cellular processes such as glucose metabolism, apoptosis, cell proliferation, transcription, andcell migration. Using the PamGene12 Kinome Array platform, we identified AKT as a signaling node inAD dorsolateral prefrontal cortex (DLPFC). We hypothesize that AKT activity is altered in this disorder.Using the DLPFC from postmortem brain, the expression and the activity of AKT isoforms (AKT1,AKT2, AKT3) will be assayed in AD and SCZ using biochemical activity assays plus and minus specificAKT inhibitors. AKT expression will also be measured at the transcript and the protein level.The results of these experiments are expected to serve as validation of the Kinome Array platformas well as provide valuable insight into how AKT is perturbed in these neuropsychiatric disorders. Tofurther probe AKT's role in AD and SCZ, this research will also encompass interacting biochemicalpathways in which AKT is a key player, which will provide novel pathophysiological insights into thesediseases.

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Poster

P334. Stroke and Mechanisms of Abnormal Upper Limb Movement

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P334.01

Topic: C.09.Stroke

Title: Quantitative assessment of the perception of movement in humans

Authors: *E. CARRANZA, A. BOOS, M. CAPOGROSSO, G. F. WITTENBERG, E. PIRONDINI;
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Abstract: A key sensory component for volitional movement is proprioception, which relies on a sense of muscle and tendon stretch to indicate position, motion, and force. Since proprioception updates the spatial properties of body parts to plan motor commands, its loss can affect all

aspects of force production, positioning and dynamics during voluntary movement. Proprioception is typically tested passively by assessing the perception of limb position in space. However, proprioceptive afferents largely convey information on movement and movement velocity that are modulated during active movement. Here we developed a battery of robotic tasks to assess position sense, kinesthesia, and force and evaluate proprioception during volitional movement. These tasks were designed to obtain metrics that distinguish proprioceptive and motor control skills. To isolate motor from proprioceptive skills, we evaluate the participant's ability to reach to targets while moving in a horizontal plane. For this, the subject is asked to perform a visually guided reaching (VGR) task in which eight targets have to be reached quickly and accurately. Three targets with the straightest and smoothest movement trajectories are chosen for further testing. With vision of the arm occluded, subjects are asked to actively reach these targets and report when they perceive they have been reached. The shift between the position reached and the real target location is then calculated to assess active sense of position. For the second task, we studied the participant's active kinesthetic sense. First the robot moves the participants affected limb, then the participant is asked to perform a reaching or pulling motion matching the sensed speed. The subject's ability to match the speed, direction, and length of the dynamic target is then measured for each trial. To determine the proprioceptive and motor control components of movement, each task is evaluated with and without visual feedback. We tested these tasks in able-bodied subjects and a group of stroke participants. The latter showed increased errors when trying to match the target's final location in contrast to healthy participants, but not during VGR tasks. In the kinesthetic task, stroke participants show impaired control trying to match the speed of the dynamic target only when relaying on proprioception (without visual feedback). Our results demonstrate that our tasks can reliably dissociate motor control from proprioception during volitional movements. These tasks will help to understand the importance of active proprioception in motor control and support the development of novel stroke rehabilitation techniques.

Disclosures: E. Carranza: None. **A. Boos:** None. **M. Capogrosso:** None. **G.F. Wittenberg:** None. **E. Pirondini:** None.

Poster

P334. Stroke and Mechanisms of Abnormal Upper Limb Movement

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P334.02

Topic: C.09.Stroke

Title: Spinal cord stimulation requires inhibitory mechanisms to enable voluntary motor control after paralysis

Authors: J.-M. BALAGUER[1], M. CAPOGROSSO[2];
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Abstract: Epidural spinal cord stimulation (SCS) improves voluntary motor control after spinal cord injury (SCI). It has been proposed that SCS increases the excitability of spinal motoneurons thus amplifying residual supra-spinal inputs that enable volitional movement. However, no studies to date have directly measured how SCS interacts with residual voluntary commands within the spinal cord. Understanding these mechanisms would be critical to optimizing SCS for clinical use. Addressing this interaction can be lengthy and costly through experimental models without neurophysiological guidance. In silico models can unveil latent insights optimizing the experimental design. Thus, we implemented a realistic computational model of spinal motoneurons. Our model included inputs both from proprioceptive afferents that were recruited by SCS and residual excitatory cortico-spinal axons, which are rarely completely abolished after SCI. We simulated subthreshold or suprathreshold SCS pulses as synchronous synaptic inputs and subthreshold cortico-spinal inputs as stochastic. Simulations revealed two types of interactions as a function of the stimulation amplitude. Natural asynchronous cortico-spinal inputs increased the motoneurons membrane potentials, amplifying subthreshold postsynaptic potentials induced by SCS. However, this led to firing rates that were too low to generate movement. In contrast, suprathreshold SCS completely dominated motoneurons responses, uninfluenced by cortico-spinal inputs. Our results contradicted the leading hypothesis for the generation of voluntary movement. Residual supra-spinal inputs increased the responsiveness of motoneurons to subthreshold SCS responses, contradicting the leading hypothesis for the generation of voluntary movement through SCS. Conversely, when SCS parameters delivered a suprathreshold response, our simulations suggest that this modulation may not be sufficient to explain the experimental results of SCS after SCI. Therefore, we now propose that mechanisms related to the regulation of spinal excitability such as presynaptic inhibition may play a critical role in this context. In particular, residual supra-spinal axons could exploit surviving GABAergic interneurons to reduce unwanted motoneuron firing caused by suprathreshold SCS pulses. In this work, we present these hypotheses in the form of simulations of alternative spinal circuit structures exploring the effects of presynaptic inhibition during SCS. We believe that future work should start from our proposal to validate and explore the role of voluntary sensory gating during SCS rather than direct cortico-spinal excitation.

Disclosures: J. Balaguer: None. **M. Capogrosso:** None.

Poster

P334. Stroke and Mechanisms of Abnormal Upper Limb Movement

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P334.03

Topic: C.09.Stroke

Title: Spinal Cord Stimulation of the Cervical Spinal Cord Improves Both Gross Strength and Fine Force Control of the Upper Limb in Post-Stroke Hemiparesis

Authors: *E. SORENSEN[1], M. P. POWELL[2], N. VERMA[5], E. CARRANZA[1], A. F. BOOS[3], L. E. FISHER[4], D. J. WEBER[5], P. GERSZTEN[2], G. F. WITTENBERG[3], E. PIRONDINI[4], J. W. KRAKAUER[6], M. CAPOGROSSO[2];
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Abstract: In strokes involving subcortical brain structures, damage to the corticospinal tracts often leads to severe deficits of volitional motor control. There is currently no effective therapy for such cases. Importantly though, spinal cord circuits responsible for movement remain intact and supraspinal connections are not completely severed. Here we seek to understand if these residual connections are sufficient to synergistically interact with epidural spinal cord stimulation (SCS) to restore volitional upper limb motor control. In a participant with chronic stroke of the internal capsule, we implanted two percutaneous linear SCS leads spanning the C4 to T1 dorsal roots. Continuous stimulation was delivered through these leads to evoke motor potentials in the deltoids, biceps, triceps, or forearm. Compared to the no-SCS condition, we observed marked improvements in force production when the participant was asked to produce isometric force of the shoulder, elbow, and wrist with some joints demonstrating over a two-fold increase when volitional effort was paired with SCS. Notably, SCS alone did not induce any measurable force output. This suggests that it is the interaction of stimulation and supraspinal inputs that enables the observed increase in strength. We then asked the participant to perform an isometric roadway test, in which they were tasked with following a thresholded pathway to gradually increase, sustain, and decrease force in a controlled manner. The maximum force required to complete the test was within the ability of the participant without stimulation. The participant accurately followed the roadway during SCS, demonstrating that volitional input can regulate stimulation induced excitation. Furthermore, the participant's performance during SCS compared to no-SCS improved dramatically as assessed by a significant improvement in accuracy. This suggests that SCS improved not only gross muscle strength, but also fine force control. We have begun investigating how an individual with hemiparesis might regulate motor outputs during continuous stimulation in this manner by directly comparing evoked muscle responses during continual passive motion as opposed to matched volitional isokinetic motion. This should demonstrate effects of SCS and volitional muscle activation independently from sensory dependent modulation of motoneuron excitability. In conclusion, we showed that SCS not only improves force production, but also fine force control in a participant with moderate post-stroke hemiparesis. In the future we aim to investigate the mechanisms of volitional motor control during SCS in subjects with stroke.

Disclosures: E. Sorensen: None. **M.P. Powell:** None. **N. Verma:** None. **E. Carranza:** None. **A.F. Boos:** None. **L.E. Fisher:** None. **D.J. Weber:** None. **P. Gerszten:** None. **G.F. Wittenberg:** None. **E. Pirondini:** None. **J.W. Krakauer:** None. **M. Capogrosso:** None.

Poster

P334. Stroke and Mechanisms of Abnormal Upper Limb Movement

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P334.04

Topic: C.09.Stroke

Title: Cervical Spinal Cord Stimulation Restores Synergistic Activation of Upper Limb Muscles During Reaching and Grasping Task in Patient with Post-Stroke Hemiparesis

Authors: *N. VERMA[1], M. P. POWELL[3], E. SORENSEN[4], E. CARRANZA[4], S. J. PARK[2], A. BOOS[5,7], L. FISHER[4,6,8], G. F. WITTENBERG[5,7], P. GERSZTEN[3,7], E. PIRONDINI[6], M. CAPOGROSSO[3,4], D. WEBER[1];
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Abstract: Stroke is the 5th leading cause of death and the leading cause of permanent disability in the United States. There are more than 7 million stroke survivors living in the US, two-thirds of whom have motor deficits. Even though physical exercise can improve motor function in this population, outcomes are highly variable across patients and a large fraction of the population is left with permanent motor deficits. Here we show that epidural cervical spinal cord stimulation (SCS) improved motor control of the arm and hand by restoring synergistic muscle activation in a person with chronic hemiparesis post-stroke. The study participant was implanted with 2 percutaneous spinal cord stimulator leads in the lateral epidural space of the cervical spinal cord for a duration of 30 days. SCS was delivered using a custom-built stimulation system that allowed stimulation on multiple contacts, either continuously or upon movement onset, detected through EMG signals. The participant performed planar and 3D reaching and grasping movements with her affected limb with and without SCS. Stimulation was applied on three monopolar contacts that were found to facilitate the recruitment of muscles at the shoulder, elbow, and hand. Tonic and phasic modes of stimulation were tested. During tonic SCS, the stimulation parameters were fixed and delivered continuously throughout the entire movement. During phasic stimulation, an increase in volitional EMG at the shoulder triggered stimulation bursts of fixed duration, linked to the phase of the motion (reach, grasp or retract) while the participant performed the task. The participant was able to perform the task faster with SCS on, than it being off, with smoother trajectories and a higher range of motion in the shoulder, elbow, and hand, leading to overall better task performance. The muscle activations were higher during the trials where the stimulation bursts were linked to the phase of movement than with continuous and no-stimulation conditions. EMG from 14 major muscles of the arm were analyzed using dimensionality reduction methods to identify patterns of correlated activity across subgroups of muscles. We found that both tonic and phasic stimulation decreased aberrant synergies and led to more typical synergistic activation of muscle groups during different phases

of the movement. These results show the potential of SCS of cervical spinal cord to improve motor control in the upper limb in people with post-stroke hemiparesis and how targeted stimulation burst linked to a specific movement phase increases its efficacy.

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Poster

P334. Stroke and Mechanisms of Abnormal Upper Limb Movement

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P334.05

Topic: C.09.Stroke

Title: Spinal cord stimulation for the restoration of upper limb motor function in post-stroke hemiparesis

Authors: *M. P. POWELL[1], N. VERMA[5], E. SORENSEN[2], E. CARRANZA[2], A. BOOS[3,6], L. E. FISHER[4,2,7], J. W. KRAKAUER[8], G. F. WITTENBERG[3,6], J. BALZER[1], P. GERSZTEN[1,6], E. PIRONDINI[4], D. J. WEBER[5], M. CAPOGROSSO[1,2];
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Abstract: Stroke is a major cause of permanent upper limb disability. Impairments caused by damage to cortico-spinal axons do not respond well to physical methods of therapy, leaving hundreds of thousands of people every year with unsatisfactory clinical outcomes. However, the spinal circuits that control movement of the arm and hand remain fully intact after stroke. Here we demonstrate that epidural electrical stimulation of the cervical spinal cord can be used to immediately improve muscle strength and motor control in a person with chronic stroke, 9 years after insult. An internal capsule lesion resulted in moderate upper limb impairment with a pre-study Fugl-Meyer Assessment for upper extremity (FMA-UE) motor score of 36/66. Two 8-contact percutaneous spinal leads were surgically implanted in the lateral epidural space spanning the C4 to T1 dorsal roots. The subject participated in a 30-day clinical trial in which we systematically evaluated the efficacy of spinal cord stimulation (SCS) to improve single joint strength and range of motion in the arm and hand, functional planar kinematics and 3D reaching movements, and performance during activities of daily living (ADL). Continuous monopolar 60 Hz stimulation was temporally interleaved between 3 electrical contacts which, combined, could elicit motor evoked potentials in the shoulder, arm, and hand. Marked improvements in isometric force production and range of motion in the shoulder, elbow, wrist, and hand were observed as

immediate effects of stimulation, with some joints demonstrating over 100% improvements in generated force. SCS-assisted planar and 3D reaching movements were smoother, faster, and more accurate than unassisted controls and allowed the subject to reach distal targets that could not be reached without stimulation. Functional movement was also evaluated using a series of common tasks ranging from drinking from a glass to cutting food with a fork and knife. In all cases, movements had better quality and amplitude with SCS. Remarkably, we observed immediate improvement in impairment, without any training as demonstrated by an SCS-assisted 13 point increase in FMA-UE score from pre-study baseline at the beginning of the trial. We also observed therapeutic effects. After the 30-day trial, the subject scored an SCS-unassisted 11 point improvement in FMA-UE score which has remained robustly elevated for at least 4 weeks after explant. Importantly, spinal stimulation did not aggravate muscle spasticity. These results demonstrate the exciting possibility that SCS can be used to improve clinical outcomes and functional independence of stroke patients with moderate upper limb impairment.

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Poster

P334. Stroke and Mechanisms of Abnormal Upper Limb Movement

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P334.06

Topic: C.09.Stroke

Title: Robotic, minimally-invasive, high-precision stereotactic approach to access subcortical structures in non-human primates

Authors: *J. HO[1,2], L. LIANG[3,2], J. M. BALAGUER[2], D. SCHAEFFER[4], A. C. SILVA[4], G. M. NAYAR[5], D. P. FIELDS[5], P. C. GERSZTEN[5], M. CAPOGROSSO[5,2,6], J. A. GONZALEZ-MARTINEZ[5], E. PIRONDINI[3,2,6];
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Abstract: Studying subcortical structures such as the internal capsule and thalamus in non-human primates has proven to be extremely difficult and requires extensive planning. There currently exist very few anatomical atlases and landmarks to provide accurate coordinates of specific brain structures, and they often vary significantly between each animal. The ROSA One® Brain surgical robot has already been implemented to provide neurosurgeons with a magnetic resonance imaging (MRI) guided and minimally invasive platform for procedures such

as stereo-electroencephalography and deep brain stimulation for patients. Here, we sought to leverage this robotic platform for non-human primate surgeries to more accurately and efficiently reach subcortical targets of interest to accelerate deep brain research. Specifically, we used the ROSA One® Robot Assistance Platform to position a stimulation probe in the internal capsule, a white matter structure in the inferomedial part of each cerebral hemisphere harboring every single cortico-spinal projection. We also placed a probe in the ventralis intermedius, a thalamic relay for incoming cerebellar projections with outgoing connectivity to the premotor and motor regions of the cortex. For this, we acquired T1-weighted MRI and CT images of the macaques in a customized plastic stereotactic frame with fiducials implanted in the skull. We subsequently co-registered these images in the ROSA robot and calibrated the robot with the implanted skull fiducials. We also utilized 3-D printed skulls of the animals from the CT images to simulate the implantation of our probes, the positions of which we later confirmed with a post-implantation CT image. Our results in the 3D printed skulls and the animal showed that our technique allowed ultra-high precise implantation of the probes (0.39 mm registration error) and electrical stimulation of internal capsule axons and the ventralis intermedius as demonstrated with intra-spinal and electromyographic recordings. In conclusion, we were able to use the ROSA robot in non-human primates as a minimally invasive, high-precision stereotactic approach to access subcortical structures of interest.

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Poster

P334. Stroke and Mechanisms of Abnormal Upper Limb Movement

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P334.07

Topic: C.09.Stroke

Support: NIH Grant F31NS095706
Sheikh Khalifa Stroke Institute

Title: Post-stroke postural abnormalities in the arm and their relationship to loss of motor control

Authors: *A. M. HADJIOSIF[1], K. KITA[1], S. T. ALBERT[2], R. A. SCHEIDT[3], R. SHADMEHR[2], J. W. KRAKAUER[1,4];
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Abstract: Abnormal resting postures are one of the most common and recognizable motor symptoms after stroke. For example, the typical hemiparetic arm posture consists of flexion at

the fingers, wrist, and elbow. This pattern appears to parallel the abnormal muscle synergies during movement, like the abnormal coupling of muscles that move the shoulder vs. the elbow joint. Whether these synergies are generated by the same mechanism as abnormal resting postures remains an open question; it might instead be that resting abnormalities are inactive during movement. Also unknown is the degree to which abnormal resting posture influences active moving and holding.
Here, we investigated resting postural abnormalities in stroke patients (N=16) and healthy, age-matched controls (N=6). We measured resting postural forces by passively moving the arm to different locations on a plane. Stroke patients showed remarkably stronger resting postural force biases in their paretic arm compared to the non-paretic arm and controls. These resting force biases were greater in more distal positions, when measured without weight-bearing arm support, and for patients with increased impairment based on the Fugl-Meyer scale - a measure primarily designed to assess abnormal synergies active during movement. These observations suggest a common mechanism underlying abnormal resting postures and abnormal movement synergies. We then examined the interplay between resting posture abnormalities and the control of reaching and holding actions in the same workspace. The same participants made repeated, arm-supported 2D reaches between 5 different targets. Patients' (unperturbed) movements were impaired compared to the non-paretic arm and controls: they were slower and took a longer path to reach the target. Since postural abnormalities may differentially affect regular vs. perturbed movement, we also examined patients' responses to perturbing forces applied during reaching; these were also impaired. When we compared unperturbed and perturbed movement performance against resting posture, however, we did not find any systematic effects of resting postural forces either across patients with different resting postural force magnitudes, nor between high- vs. low-resting postural force regions for the same patient. This suggests a dissociation between abnormal resting posture and reaching dexterity. Finally, we examined perturbations during active holding. Patients' ability to recover from these perturbations tended to improve when the resting postural force was in a direction that would resist the perturbation, suggesting that resting postural forces influence holding control in stroke.

Disclosures: A.M. Hadjiosif: None. **K. Kita:** None. **S.T. Albert:** None. **R.A. Scheidt:** None. **R. Shadmehr:** None. **J.W. Krakauer:** None.

Poster

P334. Stroke and Mechanisms of Abnormal Upper Limb Movement

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Program #/Poster #: P334.08

Topic: C.09.Stroke

Support: NIH Grant 1T32HD101395-01A1
NIH Grant 1R01NS105759-01A1

Title: Quantifying the effect of trunk postural control deficits on arm reaching in hemiparetic stroke

Authors: *K. SUVADA, J. DEOL, J. DEWALD, A. ACOSTA;
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Abstract: The trunk provides a stable base of support and facilitates interaction of the limbs with the environment. Post hemiparetic stroke, damage to descending corticospinal pathways impacts motor control and activities of daily life. In the arm, impairments include weakness, hyperactive stretch reflexes, and involuntary coupling of shoulder abduction with elbow, wrist, and finger flexion, or flexion synergy. Previous studies suggest that the flexion synergy is a result of increased reliance on reticulospinal pathways, also responsible for maintenance of posture. In the trunk, studies found excessive trunk movements, larger sway area during sitting, weakness, and altered coordination compared to controls. Yet, little is known about the role of the flexion synergy in trunk-arm coordination.This study provides evidence of the impact of stroke on trunk-arm coordination presumably due to increased use of the reticulospinal system wherein the arm and trunk are utilizing the same pathways. We designed a novel experimental paradigm that uses a haptic robotic device to create virtual environments, a motion capture system measuring trunk and arm kinematics, surface EMGs to measure trunk and arm musculature activity, and pressure mats on the seat and back to quantify trunk center of pressure.Four controls and eight stroke participants provided informed consent prior to enrolling in the study. Participants were seated with their arm coupled to the robotic device, allowing motion on a plane while supported on a virtual table or generating 25% or 50% of their maximum shoulder abduction torque (SABT). Participants were instructed to reach as fast and as far as possible for all conditions. For controls, regardless of restrained vs. unrestrained in the table (95±8 vs. 96.96±5), 25% (95 ±5 vs. 90±7), and 50% (93±3 vs. .93±4 % limb length) conditions, the reaching distances (mean±std normalized to limb length) were comparable. For stroke and trunk restrained vs. unrestrained in the table (92±9 vs. 90±8), 25% (86.±1 vs. 82±9) and 50% (86±9 vs. 84±10) conditions we saw an effect of trunk restraint. In addition, in stroke, there was increased trunk movement with the trunk unrestrained vs. restrained across conditions (mean±std mm): table (25.3±13.3 vs. 12.6±7.8), 25% (38.7± 13.6 vs. 26.8±10.8) and 50% (39.0±20.2 vs. 24.6±8.3).Results show the detrimental effect of impaired trunk control on reaching ability, compounding the effect of the flexion synergy. While additional participants are needed to show statistical significance, the data shows clear trends of decreased reaching distance during unrestrained with loading conditions and the effect of stroke on trunk-arm motor control.

Disclosures: K. Suvada: None. **J. Deol:** None. **J. Dewald:** None. **A. Acosta:** None.

Poster

P349. Animal Models of Brain Injury: New Models and Biomarkers

Location: Virtual Only

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Program #/Poster #: P349.01

Topic: C.10. Brain Injury and Trauma

Support: New Jersey Commission on Brain Injury Research, CBIR20PIL004 2RN
Osteopathic Heritage Foundation for Primary Care Research 2RN
New Jersey Commission on Brain Injury Research, CBIR19IRG025 2BW

Title: Physiological Response and Tissue Damage Following Different Depths of Impact in a Rodent Model of Mild Traumatic Brain Injury

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Abstract: Mild traumatic brain injury (mTBI) is a serious public health concern that can result in neurological and behavioral deficit. mTBI results from impact to the head and can be repetitive in nature, especially in sports and domestic violence. Our laboratory studies the effects of repetitive mTBI (rmTBI) on risky choice behavior in rodents using a closed-head controlled cortical impact (CH-CCI) model of injury and a probabilistic discounting task that assesses risk-based decision-making behavior. We found that females, but not males, display transient increases in risky choice behavior following three CH-CCI's delivered at 5.5m/s velocity and 2.5mm impact depth. These findings suggest that our injury parameters may yield marginally threshold influences on behavior that do not allow observation of the extent of rmTBI-induced effects and have prompted us to expand our model to include greater depths of injury. In the present work we subjected rats to a series of three repetitive fixed velocity impacts at depths of 2.5mm, 3.0mm, or 3.5mm. The goal was to compare physical indicators of injury in male and female rats following different depths of injury. We posited that these indices of injury would be more prominent as injury depth increased. The survival rate, righting reflex time, skull observations, and animal weights were evaluated post-injury. All animals survived all three injuries at all impact depths. We found that the average righting reflex time increased as the injury depth increased for both males and females. Males exhibited overall longer righting reflex times as compared to females at the 3.5mm impact depth. In comparison to the 2.5mm group, a greater percentage of animals in the 3.0mm and 3.5mm groups had moderate and significant skull fractures and hematomas, and a greater incidence of skull indentation. Weight gain post-injury did not differ amongst injury depths. Nissl staining showed that structural damage of tissue within the region under the impact site increased as the depth of injury increased. Studies using silver and glial fibrillary acidic protein (GFAP) stains to examine axonal injury and the astrocytic response to injury, respectively, supported these findings. The current results show that physiological measures of injury are positively correlated with depth of impact in the CH-CCI model of TBI. These data will be used to guide selection of CH-CCI impact depth in future studies designed to reveal changes in behavior following rmTBI. Based upon these findings we predict that greater depths of impact will produce greater post-injury deficits in behavioral tests of executive function and that these deficits will be greater in female vs male animals.

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Poster

P349. Animal Models of Brain Injury: New Models and Biomarkers

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Program #/Poster #: P349.02

Topic: C.10. Brain Injury and Trauma

Support: NJBIR Grant CBIR19IRG025
NJBIR Grant CBIR20PIL004

Title: Mild traumatic brain injury produces transient increases in risk taking behavior in female rodents

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Abstract: Mild traumatic brain injury (mTBI) is the most common form of TBI. Many individuals, such as athletes, often experience repetitive mTBIs over the course of their athletic careers. Consequences of mTBIs include impairments to higher order cognitive processes such as decision making. Risk-based decisions involve choices that have probabilistic outcomes. Increased risk taking behavior has been reported for TBI patients following a single TBI event; however, little information is available regarding the effects of repetitive mTBIs on risk-based decision making. Here, we examined how single and repetitive mTBI affects risk-based decision making in rodents using a well-established probabilistic discounting task (PDT). Age-matched male and female rats were trained on the PDT and then exposed to either a single or a series of three closed head control cortical impact (CH-CCI) injuries over the course of one week. Rats then returned to the PDT for testing for four weeks. We observed no changes in risky choice behavior across all four weeks of testing in male rats following either single or repetitive mTBI. In contrast, mTBIs, specifically repetitive injuries, increased risky choice behavior in female rats at one week and two weeks post injury. Increased risky choice behavior was more prominent two weeks post injury with effects being driven by a reduced sensitivity to non-rewarded outcomes following a risk-based decision, demonstrated by a decrease in lose-shift responses. When evaluated three and four weeks post injury, choice behavior was no longer significantly different from uninjured animals indicating that the observed effects of mild head injuries in female rats are transient. These results indicate that females are more susceptible to changes in risk-based

decision making following repetitive mild TBIs in comparison to males of the same age. Overall, when used together, the CH-CCI model of repetitive mTBI and the PDT are useful tools for experimentally evaluating and differentiating the effects of head injury on male vs female subjects.

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Poster

P349. Animal Models of Brain Injury: New Models and Biomarkers

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Topic: C.10. Brain Injury and Trauma

Support: VA ORD BLR&D UFR-002-18F
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University of Missouri-PAS (ZG)

Title: Low-intensity blast exposure results in glutamatergic hyperexcitability and long-term learning behavior deficits

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Abstract: Mild traumatic brain injury (TBI), frequently caused by low-intensity blast (LIB) exposure, has the highest reported TBI incidence in all military branches. Of significant concerns are the neurocognitive consequences commonly found in LIB-exposed service members and Veterans that require elucidation of their neurobiological mechanisms. Our previous studies have shown that open-field LIB exposure results in nanoscale ultrastructural abnormalities in the absence of macroscopic injury or apparent cellular damage in mice. In this study, we identified an increase in frequencies of miniature excitatory postsynaptic currents (mEPSCs) and a burst pattern of mEPSCs recorded in hippocampal CA3 neurons, a region involving synaptic plasticity, cognitive function, and memory processes in LIB-exposed mice at day-1 and -7 post-injury.

Blocking NMDA receptors with a specific antagonist significantly reduced LIB-induced mEPSC increases. Additionally, LIB increased the numerical density of asymmetric excitatory synapses in the hippocampus. Long-term cognitive function was assessed 90 days post-LIB exposure using an automated home-cage monitoring system with minimal human interference. By monitoring mouse behavior for 96 consecutive hours, we identified dynamic patterns of discrimination learning and cognitive flexibility deficits in LIB-exposed mice compared with sham controls. Bioinformatic analysis of quantitative global-proteomes of hippocampi showed alterations of synaptic proteins associated with learning deficits in LIB-exposed mice in the chronic phase. Our findings suggest that hyperexcitability caused by excessive glutamatergic synaptic inputs in the hippocampus is involved in long-term cognitive dysfunction following LIB. This study provides a platform to examine therapeutic strategies to prevent or mitigate long-term cognitive disorders caused by LIB.

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Poster

P349. Animal Models of Brain Injury: New Models and Biomarkers

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Program #/Poster #: P349.04

Topic: C.10. Brain Injury and Trauma

Support: NINDS R21NS120022
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The Brain Injury Association of America's Brain Injury Research Fund

Title: Traumatic brain injury leads to disrupted REM sleep in juvenile rats

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Abstract: Traumatic brain injury (TBI) disrupts sleep, which impairs recovery and increases risk for long-term morbidities. In the clinic and in our laboratory, TBI evokes sleep-wake disorders in adults, but few studies have focused on sleep in pediatric TBI survivors. Sleep and wakefulness require a fine interplay between homeostatic and circadian drive, and in juveniles, sleep plays a critical role in development. Moreover, endocrine disruptions, or changes to hormone production and release, occur after TBI in adults and children. However, little is known about the interplay between TBI-induced endocrine disruptions and sleep-wake disorders in pediatrics. Here, we

implanted telemetry devices to obtain EEG and EMG signals in freely moving juvenile rats (post-natal day 35) subjected to a diffuse TBI (midline fluid percussion injury) or control sham surgery. Recordings were obtained and sleep-wake behaviors (active wake, quiet wake, non-rapid eye movement (NREM), rapid eye movement (REM)) were determined for 48hrs post-injury. In a separate cohort of rats, we utilized non-invasive piezoelectric sleep cages to measure long-term (i.e., 28 days post-injury) sleep-wake disturbances and TBI-induced changes to hormone profiles at various time points post-injury. We hypothesized that brain-injured rats would display altered NREM and REM sleep post-injury compared to uninjured sham rats, and that these sleep disturbances would be mediated by changes in specific hormones. Juvenile rats subjected to diffuse TBI had enhanced quiet wakefulness ($p < 0.05$) with no change to active wakefulness during the first 48hrs post-injury compared to shams. Interestingly, juveniles subjected to TBI displayed a significant decrease in REM sleep ($p < 0.05$) compared to controls but had comparable levels of NREM sleep. REM sleep is involved in the regulation of emotions and memory consolidation; thus, disrupted REM sleep may lead to the affective and cognitive symptoms experienced by pediatric TBI survivors. Currently, we are investigating growth hormone (GH) as one potential hormonal mechanism behind the observed changes in sleep. GH levels in juveniles are high as a result of development and GH also contributes to sleep regulation. Our previous work has shown that TBI leads to GH deficiency in the pediatric population, while others have shown that GH and sleep are interrelated. As such, our ongoing studies investigate TBI-induced changes in GH as a mechanism underlying post-TBI sleep disorders in juveniles. A comprehensive understanding of these underlying mechanisms will facilitate the development of effective pharmacotherapies and improve quality of life in survivors of pediatric TBI.

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Poster

P349. Animal Models of Brain Injury: New Models and Biomarkers

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Topic: C.10. Brain Injury and Trauma

Support: 5T32HL007953-20

Title: Measured intrinsic cellular properties and afferent synaptic activity are not the cause of reduced orexin neuron firing at baseline activity levels following mild traumatic brain injury

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Abstract: Sleep disorders are a common symptom following Traumatic Brain Injury (TBI), with 30-70% of patients experiencing some type of disrupted sleep after TBI. Patients that suffer a TBI have a higher rate of sleep disorders than occur by chance in the general population, which suggests that TBI may contribute to sleep disorders. Surprisingly, alterations in sleep circuitry caused by TBI are understudied. One of the critical regulators of sleep circuitry is the neuropeptide orexin, which promotes wakefulness. It is known that the production of orexin is reduced after TBI, but the mechanism underlying this reduction remains unknown. Our lab previously published evidence that mild TBI (mTBI) does not destroy orexin neurons, but can still cause sleep disruptions in mice. After mTBI, mice displayed an inability to maintain wakefulness for long periods of time. These injured mice also showed reduced orexin activity as measured with staining for the immediate early gene cFos. cFos staining indicates that orexin neurons fire fewer action potentials after injury but cannot explain if this reduced activity is due to changes in orexin neurons themselves or alterations to afferent signaling. To specifically elucidate the source of reduced orexin activity after injury, we recorded from orexin neurons using whole cell patch clamp electrophysiology. We used the Lateral Fluid Percussion Injury method on 6-8 week old mice to induce a mild TBI. Sham animals were used as controls and had the same procedures and anesthesia except for the final concussive injury. 1 week after injury, the animals were sacrificed and acute brain slices were cut that contained orexin neurons. Using current clamp recordings, we found that the action potential threshold was reduced in orexin neurons following injury, which would cause increased excitability. Using voltage clamp recordings, we found no differences in the afferent excitatory currents or the afferent inhibitory currents on orexin neurons after mild TBI. These results demonstrate that altered intrinsic excitability or afferent excitatory neurotransmission do not underlie the reduction in orexin activity induced by mTBI. Further exploration of these neural circuits is needed to determine the underlying mechanism(s).

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Poster

P349. Animal Models of Brain Injury: New Models and Biomarkers

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Topic: C.10. Brain Injury and Trauma

Support: NIH Grant P20GM109098
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West Virginia University Clinical and Translational Science Institute

West Virginia University Experimental Stroke Core
American Heart Association

Title: Mild traumatic brain injury cerebrovascular function and exacerbates stroke outcomes in mice

Authors: *B. WHITEHEAD, R. VELAZQUEZ-CRUZ, *K. KARELINA, Z. WEIL;
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Abstract: Traumatic brain injuries (TBI) can produce long term disability and also impair cerebrovascular health. Importantly, TBI is also associated epidemiologically with increased incidence of ischemic stroke and our lab has demonstrated that mild TBI one week prior to an ischemic stroke leads to worsened functional and pathophysiological stroke outcomes in mice. Due to the links among TBI, stroke, and cerebral vascular function, we hypothesized that TBI-induced cerebrovascular impairments mediate this phenomenon of enhanced vulnerability to stroke. To test this hypothesis, we performed mild closed-head TBI, or an equivalent control procedure and collected tissue seven days later. In separate cohorts of mice, we assessed cerebrovascular function via pressure myography on isolated middle cerebral arteries, collected brains for immunohistochemical (IHC) analysis of pericyte and endothelial cell interactions, or performed a middle cerebral artery occlusion (MCAO). Cerebrovascular blood flow was assessed via laser speckle flowmetry 1 hour prior to and 3 hours post MCAO. Five days after MCAO, mice were assessed for functional recovery and infarct volume via 2,3,5-Triphenyltetrazolium chloride (TTC) staining. Results from the pressure myography revealed a decrease in middle cerebral artery dilation when exposed to acetylcholine in TBI mice compared to controls ($p<0.001$). IHC analysis revealed a decrease in pericyte coverage of vasculature in TBI mice compared to control animals ($p<0.05$). Laser speckle flowmetry results show a post-stroke decrease in blood flow in TBI mice compared to controls ($p<0.05$). Additionally, motor impairments were seen post-stroke in TBI mice compared to shams ($p=0.033$) and had significantly larger infarcts following TTC staining ($p<0.001$). Pressure myography results and IHC analysis suggest a role for dysfunction of pericyte-endothelial cell interactions in regards to cerebrovascular function, while the laser speckle flowmetry data show deficiencies in post-stroke reperfusion after TBI. Taken together, our data suggest that TBI impairs the function of cerebral vessels and results in poor blood flow during reperfusion, implicating TBI-induced impairments in the cerebral vasculature in post-injury vulnerability to subsequent ischemia.

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Poster

P349. Animal Models of Brain Injury: New Models and Biomarkers

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Topic: C.10. Brain Injury and Trauma

Support: DoD Grant W81XWH-18-1-0166
DoD Grant W81XWH-18-1-0167

Title: Posthemorrhagic hydrocephalus causes deficits in gait and cognition and loss of the essential motile cilia transcription factor YAP

Authors: *S. ROBINSON, **Y. KITASE**, M. FOUDA, S. HAMIMI, L. JANTZIE;
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Abstract: Young adults who suffer intraventricular hemorrhage from vascular lesions are prone to develop hydrocephalus. Hydrocephalus contributes to chronic neurological deficits beyond the need for cerebrospinal fluid (CSF) diversion. Hydrocephalus is more likely to develop in the setting of systemic inflammation. We hypothesized that intraventricular hemorrhage (IVH) and hydrocephalus would lead to deficits in gait and cognition in a preclinical model of posthemorrhagic acquired symptomatic hydrocephalus (ASH). We also predicted that this would occur with loss of the transcription factor YAP (yes-associated protein), which is modulated by inflammation and essential for ependymal motile cilia maturation. On postnatal days 21 (P21) and P23 rats received 3mg/kg lipopolysaccharide (LPS) or sterile saline i.p. On P25, under anesthesia, rats received bilateral intraventricular injection of lysed littermate red blood cells (IVH) or sterile saline. Both sexes were used. On P42, rats underwent computerized gait analysis, and began cognitive testing with a touchscreen platform. qPCR on ependyma was performed for YAP. Group differences were compared Mann Whitney test, with $p<0.05$ considered significant. Results indicate that compared to controls, rats with ASH spent less time in propel, stance and stride stages of gait. They also had shorter stride length, and increased stride frequency (n=5, all $p<0.05$). Passing rates were similar for ASH rats (n=26) and control rats (n=14) for visual discrimination. They made a similar number of errors, and required a similar number of correction trials, showing that ASH rats could complete touchscreen testing. During a Reversal learning paradigm, ASH rats committed more errors ($p=0.0007$) and required more correction trials ($p=0.03$) compared to controls, consistent with poor cognitive flexibility. YAP mRNA levels were reduced in ASH ependyma (n=7, $p<0.01$). Acquired posthemorrhagic hydrocephalus causes deficits in gait and cognition. CSF dynamics are complex and reflect changes in choroid plexus secretion, ependymal motile cilia propulsion and glymphatic reabsorption. Clarifying functional outcomes and molecular mechanisms will lead to effective treatments that are not dependent on surgery.

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Poster

P349. Animal Models of Brain Injury: New Models and Biomarkers

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Program #/Poster #: P349.08

Topic: C.10. Brain Injury and Trauma

Title: A novel in vivo mouse mild repeated blast injury model with unrestrained head and its application in an Aldh2*2 disease model.

Authors: *Z. ZHANG[1], S. HERR[1], T. BEAUCLAIR[1], S. SUN[1], N. RACE[1], R. SHI[2];
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Abstract: Blast-induced traumatic brain injury (bTBI) is characterized by an explosive overpressure wave traveling across the brain, resulting in acceleration-deceleration shearing forces that lead to tissue destruction. Due to the complex and heterogeneous nature of bTBI and the limitations of human studies, a reliable and clinically relevant animal model is needed to deepen our understanding of its underlying mechanism. The shock tube pneumatic blast model has the practical advantage to be performed in a confined laboratory space, with less variability compared to open-field chemical explosions. Damage to the rest of the animal's organs can be minimized using a sealed body shield, isolating blast injury in the brain. While our current model has predominantly been used for rats, we have recently modified and adapted it for investigating mbTBI in mice. Multiple parameters including the thickness of polyester membrane, the distance between animal's head and the shock tube ending, and the restraint status of the head (flexible vs fixed), were adjusted to accommodate mice based on the mortality, morbidity, and various pathological markers in the brain. The pressure wave profile was recorded using a piezoelectric transducer and analyzed by a custom-made LabVIEW program, which demonstrated a characteristic Friedlander waveform. Using such model, we have shown a significant increase of reactive aldehyde acrolein adducts, an established biomarker of oxidative stress and inflammation, 2 days post repeated mbTBI in mice carrying the aldehyde dehydrogenase 2 mutation (*Aldh2*2*), an enzyme known to detoxify acrolein. Consistent with our hypothesis, acrolein adducts elevation was less prominent in wildtype mice. Further, the elevation of acrolein was observed in mice with unrestrained flexible heads, but significantly less than in mice with restrained fixed heads during the blast, implying that allowing head movement during injury could result in more severe brain injury when compared to restrained or fixed head positions. Taken together, this data suggests that, similar to our rat mbTBI model, the newly established mouse mbTBI model can also elicit secondary injury indicated by the elevation of lipid peroxidation product acrolein, demonstrating that it is a suitable rodent bTBI model for studying post-mbTBI primary and secondary injuries. In addition, our study also indicates the critical role of ALDH2 in suppressing acrolein following mbTBI. This mouse mbTBI model will enable us to further elucidate the molecular mechanism of brain damage, particularly those related to the secondary injury mechanisms, by taking advantage of various available transgenic mouse model.

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Poster

P349. Animal Models of Brain Injury: New Models and Biomarkers

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Program #/Poster #: P349.09

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant NS093570

Title: The absence of NEP-like amyloid-beta degrading enzymes improves motor/memory performance post brain injury in mice

Authors: *R. A. MARR[1], K. C. MAIGLER[1], T. J. BUHR[1], C. S. PARK[1], S. A. MILLER[2], D. A. KOZLOWSKI[3];
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Abstract: Traumatic brain injury (TBI) increases the risk for neurodegenerative diseases including Alzheimer's disease (AD). Furthermore, both human and animal model data indicate that the amyloid-beta (Aβ) peptide and its production machinery are upregulated by TBI. Considering the clear link between chronic Aβ elevations and AD, Aβ may have a role in the processes following brain injury. Proteases, including the neprilysin (NEP)-like enzymes, are key mediators of Aβ clearance and so may influence the susceptibility to pathology post-brain injury. We used a mouse model expressing normal human Aβ-precursor protein (APP) under its natural transcriptional regulation. These transgenic mice were also crossed with mice deficient in the NEP or NEP2 Aβ degrading enzymes and these lines were exposed to repeated closed-head TBI. Our results show that the presence of the human form of Aβ (not overexpressed or carrying familial-AD mutations) did not affect motor (Rotarod) and spatial learning/memory deficits (Morris water maze) post-injuries. However, NEP and NEP2 deficiency was associated with protection from motor (NEP or NEP2 deficient) and spatial learning/memory deficits (NEP deficient only) at acute time points post injury (*J Neurotrauma* 38(5):665-676). These data suggest that NEP and NEP2 peptide substrates are protective at acute time points post injury and a potential beneficial role for Aβ during the early response to brain injury.

Disclosures: R.A. Marr: None. **K.C. Maigler:** None. **T.J. Buhr:** None. **C.S. Park:** None. **S.A. Miller:** None. **D.A. Kozlowski:** None.

Poster

P349. Animal Models of Brain Injury: New Models and Biomarkers

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P349.10

Topic: C.10. Brain Injury and Trauma

Support: CNRM-70-9922
USU-PAT-74-3439
CDMRP W81XWH-13- 2-0018

Title: Actigraphic evidence of persistent sleep disruption following repetitive mild traumatic brain injury in a gyrencephalic animal model

Authors: S. C. SCHWERIN[1], A. OBASA[1], T. HAIGHT[2], M. RAY[3], *S. L. JULIANO[1]; [1]APG, Uniformed Services Univ. of Hlth. Grad. Program In Neurosci., Bethesda, MD; [2]Ctr. for Neurosci. and Regenerative Med., Bethesda, MD; [3]APG, USUHS, Bethesda, MD

Abstract: Disrupted sleep is prevalent after traumatic brain injury (TBI) and characterized by insomnia, increased awakenings, reduced sleep efficiency, and daytime somnolence. Sleep disruption frequently exacerbates other issues, such as depression, anxiety, stress, and pain. An animal model exhibiting disturbed sleep/activity patterns after brain injury will be useful to study neuropathological mechanisms of sleep disruption and to test therapeutics. We assessed sleep/activity patterns in adult male ferrets at baseline and following TBI. Ferrets wore an ActiGraph, which we evaluated at baseline, 4-5 weeks post injury, and 4 and 6 months post injury. The injured animals received repeated blast exposure and a rotational brain injury over a period of 2 weeks; a subset of animals also received stress prior to and concurrent with the brain injuries. At baseline, actigraphy patterns in ferrets show distinct clusters of active and inactive periods; the inactive periods correspond to sleep. At 4 weeks and 4 months post injury the ferrets in both the Injury Alone (I) and Injury + Stress (I+S) groups showed fractured and dispersed sleep/activity patterns. We calculated the frequency of high activity bout lengths and found that after injury, short bout lengths (1-5 minutes) were much more frequent than at baseline, while many fewer long high activity bout lengths (16-60 minutes) occurred. The percent of time spent inactive (asleep) decreased during the night for the injured group at 4 and 6 months post injury, while the percent of time spent in high and low activity bouts increased, reflecting reduced nighttime sleep efficiency. During the daytime, the percent time spent highly active decreased in the I+S animals, which may reflect increased daytime sleepiness. We further investigated changes in high activity using Loess analyses. Both the I and I+S groups spent significantly less time in high activity compared to the Sham group at 4 weeks and 4 months post injury. Overall our analysis shows that fragmented sleep persists until at least 6 months post repetitive mild TBI with and without stress.

Disclosures: S.C. Schwerin: None. **A. Obasa:** None. **T. Haight:** None. **M. Ray:** None. **S.L. Juliano:** None.

Poster

P353. Traumatic Brain Injury: Human Studies: Biomarkers

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P353.01

Topic: C.10. Brain Injury and Trauma

Support: Indiana Spinal Cord Brain Injury Research Fund 43545
Zeiss Equipment Grant

Title: Retinal thickness changes may be detectable as objective biomarkers in the acute phase of concussions

Authors: L. WILKINS[1], T. R. ZUIDEMA[2], L. M. FERRIS[3], B. J. KING[2], ***N. L. PORT**[2]; [1]Dept. Psychological and Brain Sci., [2]Sch. of Optometry, Indiana Univ., Bloomington, IN; [3]Dept. of the Air Force, San Antonio, TX

Abstract: Purpose: Concussions are a growing public health concern. Photophobia and blur are two common symptoms of concussion, yet no objective visual biomarkers of concussion currently exist in practice. Optical coherence tomography (OCT), a microscopic tool for imaging in vivo human retina, is commonly used to detect neurodegenerative conditions and may serve as an objective biomarker for acute concussions. In this matched cohort pilot study, we analyzed visual symptoms and investigated whether retinal changes are detectable in the acute and/or recovery phase of concussion.
Methods: Acute concussion (≤ 72 hours) and recovery (2 weeks, 8 weeks, 6-18 months) data were collected on a total of n = 39 NCAA Division I collegiate athletes; n = 19 concussed and n = 20 age, sex, and sport-matched controls. At each visit, retinal OCT and a visual function survey were conducted. An ANOVA was conducted to analyze significance and descriptive statistics, including mean, variance, and effect size between injury groups measured with Cohen's d, to evaluate the behavioral and OCT data.
Results: Only one question on the visual function questionnaire (To what extent does sunlight currently bother you when you are not wearing sunglasses?) at 24-72 hours significantly differentiated concussed from non-concussed ($p < 0.05$). Retinal OCT data of the macula showed small, yet significant differences between concussed and control athletes in the outer superior macular nerve fiber layer (NFL) ($d = 0.05$, $p = 0.0456$), outer temporal macular NFL ($d = 0.58$, $p = 0.0226$), and the outer inferior macular ganglion cell layer ($d = 0.22$, $p = 0.0490$) between initial acute and 8-week recovery time points. No other macula or optic nerve head sectors were significant ($p > 0.05$).
Conclusion: Retinal OCT measurements may show promise as objective biomarkers for assessing acute concussion and recovery in collegiate athletes. Consistent with existing literature, photosensitivity is commonly reported and graded more severe in athletes suffering from acute concussion. These findings highlight a possible pathophysiological relationship between vision

and concussion. Further study is warranted, investigating the potential use of commercially available OCT devices for concussion assessment and management.

Disclosures: L. Wilkins: None. **T.R. Zuidema:** None. **L.M. Ferris:** None. **B.J. King:** None. **N.L. Port:** None.

Poster

P353. Traumatic Brain Injury: Human Studies: Biomarkers

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P353.02

Topic: C.10. Brain Injury and Trauma

Support: National Collegiate Athletic Association

Title: Exploring associations between resting-state functional connectivity and concussion history in relatively young former collegiate football players

Authors: *S. R. WALTON[1], J. R. POWELL[1], W. YIN[1], B. L. BRETT[2], W. LIN[1], A. S. NENCKA[2], M. LIU[1], H. GUAN[1], M. A. MCCREA[2], K. M. GUSKIEWICZ[1], K. S. GIOVANELLO[1];
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Abstract: *PURPOSE*: Sport-related concussions (SRCs) and cumulative head impact exposure via contact sport participation may be risk factors for later-life neurodegenerative changes in former football players. Studies in typical aging, neurodegenerative disease, and acute SRC have reported similar patterns of altered resting state functional connectivity (rsFC) between large-scale brain networks (e.g., neural signatures of dysfunction). The purpose of this study was to investigate whether history of SRC was associated with similar neural signatures in rsFC among relatively young former football players removed from sport for at least 15-years.
METHODS: Former collegiate football players (*n*=54; aged 37.9±1.5 [range 35-41] years; body mass index [BMI]=30.8±4.2 $kg \cdot m^{-1}$) enrolled in this study reported 0 or 1 ("Low"; *n*=24) or 3+ ("High"; *n*=30; median[IQR]=6.5[4-10]) lifetime SRCs. Multisite resting-state fMRI and 3D T1-weighted images were acquired via Siemens Prisma 3T (*n*=38) or GE Signa Premier 3T scanners (*n*=16). T1-weighted images were pre-processed using the standard Freesurfer pipeline; fMRI images were pre-processed using FSL. Next, rsFC was calculated via custom Matlab code utilizing the Yeo et al. (2011) 7-network functional atlas. Based on prior research, between-network rsFC (average ROI-ROI correlation values) were calculated for the whole brain (i.e., average of all between-network rsFC values) and specifically between each of the Default Mode (DMN), Dorsal Attention (DAN), and Frontoparietal Control (FPCN) networks. All rsFC metrics were compared between Low and High SRC history groups using ANCOVA models accounting

for participant age, BMI, and acquisition site with *a-priori* α=0.05.
RESULTS: There were no differences between the High and Low SRC history groups for any of the between-network rsFC measures: whole-brain F=0.666, p=0.42, η^2_p=0.013; DAN-FPCN F=0.185, p=0.67, η^2_p=0.004; DAN-DMN F=0.065, p=0.80, η^2_p=0.001; DMN-FPCN F=0.011, p=0.92, η^2_p=0.000.
CONCLUSIONS: Neural signatures of altered between-network rsFC differences that have been observed in studies of typical and pathological aging, as well as acute SRC, were not observed in the High SRC history group when compared to the Low group in this study. Although SRC history has been associated with early and chronic changes in brain function in former football players, these preliminary data suggest that such an association, if it exists, may not be detectable in relatively young individuals.

Disclosures: S.R. Walton: None. **J.R. Powell:** None. **W. Yin:** None. **B.L. Brett:** None. **W. Lin:** None. **A.S. Nencka:** None. **M. Liu:** None. **H. Guan:** None. **M.A. McCrea:** None. **K.M. Guskiewicz:** None. **K.S. Giovanello:** None.

Poster

P353. Traumatic Brain Injury: Human Studies: Biomarkers

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P353.03

Topic: C.10. Brain Injury and Trauma

Title: High-frequency specific spreading depression in EEG of traumatic brain injury patients

Authors: *A. L. SCHWAMB[1], M. E. LOE[1], J. H. WAGNER[1], S. KHANMOHAMMADI[1], L. N. EISENMAN[2], O. J. LAURIDO-SOTO[2], T. T. KUMMER[2], S. CHING[1];
[1]Washington Univ. in St. Louis, St. Louis, MO; [2]Dept Neurol., Washington Univ. Sch. Med., Saint Louis, MO

Abstract: In spectral analysis of continuous EEG recordings from 32 patients with traumatic brain injury (TBI), we observed a novel form of electrophysiological depression. This depression was characterized by a gradual loss of power above the spectral edge, while the below-edge power remained constant (High-frequency specific spreading depolarization, HFSSD). The time-frequency spectrograms of the TBI patients we studied are generally characterized by dominant and stereotypical low frequency power below 10Hz, with diffuse and unstructured power at higher frequencies. In HFSSD, this higher frequency activity becomes significantly and repetitively attenuated (-20dB) over a time-scale of 10-15 minutes. The lower frequency activity, however, remains unchanged both in power and in any banded frequency structure that exists. To quantify and automatically detect HFSSD, we developed a novel EEG signal processing pipeline consisting of a combination of K-means clustering and Kalman smoothing. First, we used K means clustering and Kalman filter smoothing to define a set of discrete spectra that constitute

the overall spectrogram. From moment to moment, the spectrogram is defined as belonging to one of these discrete members. Sixteen canonical exemplars of HFSSD from nine patients were identified based on the aforementioned characteristics. We then used a supervised learning approach to match comparable time epochs from all 32 patients, defining these epochs as HFSSD.For the automatically detected HFSSD, post-hoc analysis of the spectrograms confirmed the same above spectral-edge loss of power as the exemplars. In 26 out of the 32 patients studied, this phenomenon localized to the area of injury (81.25%). Moreover, in these 26 patients, this phenomenon exhibited diffusive spatial spread to surrounding EEG channels, suggesting underlying cortical propagation. The timescale and the spreading nature of this phenomenon suggest that HFSSD could be associated with Cortical Spreading Depression (CSD). CSDs are known to localize to the area of injury and slowly spread out to other areas of the brain. In patients with TBI, CSDs are highly correlated with negative outcomes, but require invasive electrocorticography approaches to detect. An indication of CSD in EEG data could provide earlier and less invasive detection, which would improve understanding of their relationship to neurological outcomes and enable testing of treatment strategies.

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Poster

P353. Traumatic Brain Injury: Human Studies: Biomarkers

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P353.04

Topic: C.10. Brain Injury and Trauma

Title: Mild TBI-induced changes in peripheral innate immune cell populations across time

Authors: *J. SMITH[1], T. NGUYEN[2], M. JUBOORI[2], K. NAUGLE[3], F. A. WHITE[4]; [1]Med. Scientist Training Program, Indiana Univ. Sch. of Med., Indianapolis, IN; [2]Anesthesia, [3]Kinesiology, Indiana Univ., Indianapolis, IN; [4]Anesthesia, Indiana Univ. Sch. Med., Indianapolis, IN

Abstract: Mild TBI-induced changes in peripheral innate immune cell populations across time Jared Smith[1,2,3], Tyler Nguyen[2,3], Mohammed Juboori[2], Kelly Naugle[4], Fletcher A. `White[2,3]· [1]Medical Scientist Training Program, School of Medicine, Indiana University Indianapolis, Indianapolis, Indiana.· [2]Department of Anesthesia, School of Medicine, Indiana University Indianapolis, Indianapolis, Indiana. · [3]Stark Neurosciences Research Institute, Indiana University School of Medicine, Indianapolis, Indiana, USA.· [4]Department of Kinesiology, School of Health and Human Sciences, Indiana University/Purdue University, Indianapolis, Indiana.

Post-traumatic headache (PTH) is one of the most common and persistent symptoms following mild traumatic brain injury (mTBI), often disrupting recovery in civilian and military populations. Despite the high prevalence of PTH following mTBI, the pathological mechanisms underlying the development of chronic PTH's remain poorly understood. Recent publications suggest that monocytic cell subpopulations have a much larger role in the initial evolution of TBI than previously appreciated and this inflammatory response after mTBI may ultimately contribute to subacute or chronic PTH sequelae. For our study, adult mTBI patients were recruited from Level I Emergency Department Trauma Centers completed study sessions at 1-week, 1-month (sub-acute), and 4-6-months (chronic) post mTBI (n=31) including a headache survey which included a 0-10 numeric pain rating scale (NRS) to measure average intensity of headache pain. Additionally, we isolated the PBMCs for flow cytometric analysis with CD45, CD14, CD16, CD56, CX3CR1, CCR2, and TLR4. Monocytes were subdivided by CD14 and CD16 expression with high CD14 expression denoting inflammatory monocytes while high CD16 suggests anti-inflammatory (nonclassical) monocytes. Our data suggest substantial dynamic changes in monocyte subsets and natural killer cell distributions over the 4-month time period which could contribute to the clinical outcomes of PTH pain which may serve as biomarkers or as potential therapeutic targets.

Disclosures: J. Smith: None. **T. Nguyen:** None. **M. Juboori:** None. **K. Naugle:** None. **F.A. White:** None.

Poster

P353. Traumatic Brain Injury: Human Studies: Biomarkers

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P353.05

Topic: C.10. Brain Injury and Trauma

Title: Differential association between grey matter volume and S100B levels in mTBI

Authors: *S. CLIMIE[1], A. ANDRADE[2], T. ZUIDEMA[5], M. T. MOORE[3], J. M. CARLSON[4]; [1]Northern Michigan Univ. Departments of Biol. and Psychology, Marquette, MI; [3]Sch. of Hlth. and Human Performance, [4]Psychology, [2]Northern Michigan Univ., Marquette, MI; [5]Indiana Univ., Bloomington, IN

Abstract: Over the past decade, blood serum-based protein biomarkers have been explored as an objective measure of the neurobiological mechanisms of mild traumatic brain injury (mTBI), also known as concussion. One of the most utilized and researched biomarkers is a calcium binding protein known as S100B. The majority of S100B research has focused on its efficacy as a biomarker of concussion, with the findings regarding its efficacy varying. There is insufficient literature regarding the association between S100B and structural alterations in the brain following mTBI. Thus, the aim of this study was to (1) further explore the efficacy of S100B as a

biomarker of concussion as well as (2) evaluate the association between S100B and grey matter volume (GMV) alterations in concussed college athletes. Structural magnetic resonance images (sMRI) as well as blood serum samples were collected from 26 collegiate athletes. The experimental group included 13 athletes who were both diagnosed with a mTBI and recently cleared to return to play. A group of 13 athletes matched on age, sex, and sport were used as a control. Overall, there was not a significant difference in S100B levels between the concussed and control groups (t = -.29, p = .77). A voxel-based morphometry (VBM) regression analysis was performed to test for differential associations between S100B levels and regional gray matter volume in concussed and non-concussed athletes. Using a whole-brain cluster-level family-wise error (FWE) correction, the results showed a positive relationship between S100B and GMV in the insula in the mTBI experimental group. This suggests a positive relationship between the insula GMV and S100B levels following concussion. Lastly, the results showed that decreased GMV in the paracentral lobule, postcentral gyrus, and the superior parietal gyrus was negatively associated with S100B levels following concussion. Although more research is needed, this study provides initial insight into the relationship between serum S100B levels and GMV alterations in college athletes who experienced mTBI.

Disclosures: S. Climie: None. **A. Andrade:** None. **T. Zuidema:** None. **M.T. Moore:** None. **J.M. Carlson:** None.

Poster

P353. Traumatic Brain Injury: Human Studies: Biomarkers

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Program #/Poster #: P353.06

Topic: C.10. Brain Injury and Trauma

Support: Colorado Traumatic Brain Injury Trust Fund (part of the MINDSOURCE Brain Injury Network), Contract IHEA #101374

Title: Examining the relationship between perceived distress and salivary cortisol among Veteran participants with mild traumatic brain injury in a yoga trial

Authors: *M. DONOVAN[1,2], J. E. FORSTER[1,2], L. M. BETTHAUSER[1,2], C. STAMPER[1,2], M. PENZENIK[1], T. D. HERNANDEZ[1,2,5], N. BAHRAINI[1,2,3], L. A. BRENNER[1,2,3,4];
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Abstract: Mild traumatic brain injury (mTBI) was one of the leading injuries sustained by Veterans during recent conflicts, with many Service Members suffering from post-concussive symptoms long after the initial injury. Post-concussive headaches (PCH), one of the most widely

reported symptoms, can be both distressing and debilitating. Thus, there is an increased need to understand relationships between PCH, perceived distress, and underlying physiological mechanisms in this population. Recent work has supported the use of yoga-based interventions for treating stress dysregulation in a variety of populations, thus making yoga trials a setting within which to compare subjective and objective measures of distress in Veterans with PCH. Here, within the context of 75-minute yoga sessions across 8 weeks, we explored the relationship between perceived distress and cortisol in 16 Veterans (12 male, 4 female) with mTBI and long-term PCH. The Visual Analogue Scale (VAS), a validated tool for measuring subjective distress, was administered to participants immediately before and after yoga sessions over the course of the intervention. To allow for a comparison with VAS, participants also provided salivary cortisol (pre- and post-yoga) at 8 of the sessions. Mean age was 38.3 (standard deviation=7.6). Repeated measures ANOVAs were used to model VAS and cortisol change (post- minus pre-class scores) over time. There was a significant reduction in VAS scores for 5 of the 8 assessed yoga classes, with the estimated mean change ranging from -1.3 to -2.0 for these 5 classes (all $p<0.01$). Interestingly, there were no significant changes in cortisol levels during any of the assessed yoga sessions. Moreover, no significant correlations were found between VAS scores and salivary cortisol levels. When looking at how cortisol levels changed over time (i.e. over the series of 8 classes), there was a significant downward trajectory in post-yoga cortisol (β=-0.005 ug/dL, p=0.03); however, this result was no longer observed when taking pre-class cortisol into account (i.e., within yoga session cortisol change over time). Taken together, we found that subjective distress, but not cortisol was reduced during yoga classes, thus highlighting the ongoing challenges of bridging the gap between subjective and objective measures. Future research should explore additional biomarkers alongside subjective measures to better understand the mechanisms underlying perceived distress in those with mTBI and PCH.

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Poster

P353. Traumatic Brain Injury: Human Studies: Biomarkers

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P353.07

Topic: C.10. Brain Injury and Trauma

Support: 5R01NS100973-05

Title: Relationship between structural magnetic resonance imaging and the development of psychological distress in mild traumatic brain injury patients

Authors: *F. SIBILIA[1], R. M. CUSTER[1], A. IRIMIA[2,3], F. SEPEHRBAND[1], A. W. TOGA[1], R. P. CABEEN[1];

[1]USC Stevens Neuroimaging and Informatics Institute, Keck Sch. of Med., [2]Ethel Percy Andrus Gerontology Center, Leonard Davis Sch. of Gerontology, [3]Dept. of Biomed. Engineering, Viterbi Sch. of Engin., USC, Los Angeles, CA

Abstract: Traumatic brain injury is defined mild (mTBI) when patients have loss of consciousness less than 30 minutes, Glasgow Coma Scale score of 13-15 and post-traumatic amnesia less than 24 hours. Traumatic brain damages can alter different structural compartments and lead to the development of neuropsychological symptoms, that can persist overtime. This study aims to investigate the relationship between structural brain damages and psychological distress in a mTBI population, using multiple MRI-based modalities. Eighty-nine mTBI patients from the TRACK-TBI (Transforming Research and Clinical Knowledge in Traumatic Brain Injury) pilot study were considered for the analysis. Three subscales of the Brief Symptoms Inventory 18 (BSI18), namely Depression, Anxiety and Somatization, were used as outcome measures of psychological distress 6 months after the traumatic event. Glasgow Coma Scale scores were used to calculate improvement level. MRI was performed within 2 weeks post-injury. Perivascular spaces (PVS) number and volume were obtained by using an enhanced PVS contrast mask, both at a whole-brain and region-specific levels. Cortical thickness and grey matter structures volumes were calculated in Freesurfer, diffusion imaging indexes and multi-fiber tracts were extracted using the Quantitative Imaging Toolkit. The analysis was performed considering age, sex, education and post-injury improvement level as covariates. Perivascular spaces were found to be associated to somatization symptoms in the posterior cingulate, fusiform and postcentral areas. The three BSI subscales were associated to the cortical thickness of frontal-opercularis and occipital pole, putamen and amygdala volume, and corticospinal tract and superior thalamic radiation ($p < 0.05$, FDR corrected). Other significant associations were also found when the two hemispheres were analyzed separately. This study shows how perivascular, cortical and microstructural damages contribute to the onset of depression, anxiety and somatization symptoms in mTBI patients. The combination of different MRI modalities can reveal more on the brain areas that predict neuropsychological disorders. Future work will look at gender-based differences, and if such psychological symptoms persist after 6 months, considering BSI scores at more timepoints. Data and research tools used in the preparation of this article reside in and were analyzed using the Department of Defense (DOD) and National Institutes of Health (NIH)-supported Federal Interagency Traumatic Brain Injury Research Informatics System (FITBIR). Dataset identifier: [doi:10.23718/FITBIR/1419836].

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Poster

P386. Descending Modulation in Pain: Brainstem and Cortex

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P386.01

Topic: D.03. Somatosensation – Pain

Support: NIH Grant R01DA045664
NIH Grant R01MH116904
NIH Grant R01HL150566
Terman Fellowship
Firmenich Scholarship
Stanford Neuroscience Institute's Neurochoice Initiative

Title: The pain facilitatory role of Oprm1-expressing brainstem to spinal cord-projecting neurons

Authors: *Q. WANG, G. NACHTRAB, M. MOHR, J. XIONG, L. YUAN, X. CHOU, J. V. L. KING, M. A. HOROWITZ, X. CHEN;
Stanford Univ., Stanford, CA

Abstract: Persistent mechanical pain caused by inflammation or nerve injury is a debilitating clinical problem. The spinal cord-projecting neurons in the rostral ventromedial medulla (RVM^{SC} neurons) play active roles in pain facilitation. However, the underlying circuitry and molecular mechanisms remain largely unknown. Using fiber photometry, we recorded robust calcium signals from $OPRM1^{+}$ RVM^{SC} neurons when animal respond to noxious stimuli. We then genetically ablated $OPRM1^{+}$ RVM^{SC} neurons or chemogenetically inhibited these neurons in behaving mice and demonstrated their essential role in both initiation and maintenance of chronic mechanical pain caused by nerve injury and inflammation. Using retrograde transneuronal tracing, we identified presynaptic neurons that provide the excitatory input onto the $OPRM1^{+}$ RVM^{SC} neurons and examined their role in the onset and maintenance of mechanical hypersensitivity. Together, our results revealed that the activity of $OPRM1^{+}$ RVM^{SC} is required for both initiation and maintenance of mechanical hypersensitivity in mouse models of inflammatory and neuropathic pain.

Disclosures: Q. Wang: None. **G. Nachtrab:** None. **M. Mohr:** None. **J. Xiong:** None. **L. Yuan:** None. **X. Chou:** None. **J.V.L. King:** None. **M.A. Horowitz:** None. **X. Chen:** None.

Poster

P386. Descending Modulation in Pain: Brainstem and Cortex

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P386.02

Topic: D.03. Somatosensation – Pain

Support: Conacyt Mexico grant 255548

Title: Lidocaine and ketamine transiently revert the capsaicin-induced increase in functional coupling between the ongoing dorsal horn and brainstem neuronal activity

Authors: N. PLAMENOV[1], L. MORENO[1], D. CHÁVEZ[1], E. HERNÁNDEZ[1], B. ALVAREZ[1], P. REYES[1], S. GLUSMAN[1,2], ***P. RUDOMIN**[1,3];
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Abstract: We have shown in the anesthetized cat that during the state of central sensitization induced by the intradermal injection of capsaicin there is a non-random (structured) increase in the functional connectivity between the ongoing activity generated by dorsal horn (DH) neuronal populations distributed along the L4-L7 spinal segments. These changes are supraspinally modulated and transiently reverted by the systemic injection of sub-anesthetic amounts of lidocaine (Contreras-Hernández et al., J. Physiol 2018). Ketamine, a non-competitive antagonist of the N-methyl-d-aspartate (NMDA) receptors, is now being used to induce a quick and enduring relieve of depression. Since activation of NMDA receptors plays a significant role in the reconfiguration of neuronal connectivity during nociception, we wondered on the extent to which ketamine also modified the capsaicin-induced functional coupling between supraspinal and DH neuronal populations. To this end we recorded the ongoing field potentials generated in the bulbar reticular formation and midline raphe nuclei (RVM), in the DH and in the L4-L7 Cord Dorsum spinal segments. We used standard computational methods to estimate the changes produced by capsaicin, lidocaine and ketamine on the correlation as well as on the power spectra and coherence between the field potentials recorded at these sites. We found that both lidocaine and ketamine transiently changed the frequency spectra of the field potentials generated by the RVM and spinal cord neuronal populations recorded during the action of capsaicin and reduced the coherence between them. Quite interestingly, during the decoupling induced by lidocaine and ketamine the patterns of functional connectivity in the examined spinal and supraspinal neuronal populations resembled their pre-capsaicin configurations. We are now in the process of testing several de-correlating procedures to induce a functional decoupling between the spinal and supraspinal increased correlation induced by nociception.

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Poster

P386. Descending Modulation in Pain: Brainstem and Cortex

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P386.03

Topic: D.03. Somatosensation – Pain

Support: NIH AR073187

Title: Elucidating the role of testosterone in the descending pain pathway after activity-induced pain

Authors: *A. PLUMB[1], J. LESNAK[1], L. RASMUSSEN[1], K. A. SLUKA[2];
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Abstract: Females experience higher prevalence of chronic pain which emerges around the time of puberty suggesting that sex hormones play a role. We developed an activity-induced musculoskeletal pain model that produces behavioral sex differences in which females develop widespread hyperalgesia of longer duration, while males develop localized pain for shorter durations. Previously, we determined that testosterone mediates the behavior phenotype in male and female mice, and there are sex-dependent differences in expression of SERT in the RVM with females showing increased expression. The current study examined the role of RVM in mediating the testosterone-dependent behavior phenotype and SERT expression. The pain model was induced by two injections (pH 5.0 saline) into one gastrocnemius muscle combined with 6 minutes of fatiguing muscle contractions in C57/Black6 mice. Hyperalgesia was tested with withdrawal threshold of the gastrocnemius muscle using force sensitive tweezers. To determine if testosterone mediates antinociception centrally, we implanted a guide cannula in the RVM and microinjected drugs to modulate sex hormones in male and female mice. Since testosterone is converted to estrogen through the enzyme aromatase, we microinjected either an estrogen receptor antagonist (ICI182,780; 2-20uM) or an aromatase inhibitor (letrozole; 350uM-3.5mM) into the RVM. Blockade of either estrogen receptors ($p=.001$) or aromatase ($p=.046$) in the RVM resulted in contralateral hyperalgesia in male mice but had no effect in female mice ($p=.457$). To test the impact of testosterone on SERT expression, male mice were gonadectomized whereas female mice were implanted subcutaneously with testosterone pellets, and the RVM was immunohistochemically stained with a SERT antibody. Testosterone reduced SERT density in female mice ($p=.009$); however, gonadectomy in males has no effect on SERT levels ($p=.915$). Microinjection of a SERT inhibitor into the RVM reversed ipsilateral hyperalgesia in both male ($p=.022$) and female ($p=.041$) mice but required a higher dose to produce the same degree of analgesia in females. Our data suggests that males convert testosterone in the RVM to estradiol through aromatase to protect from development of widespread hyperalgesia. On the other hand, estrogen receptors in the RVM do not play a role in development of widespread hyperalgesia in female mice. The increased SERT expression in females is reduced by treatment with testosterone; however modulation of testosterone in males does not affect SERT expression. Understanding the sexual dimorphism in pain will lead to an optimization of individualized pain treatment.

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Poster

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Program #/Poster #: P386.04

Topic: D.03. Somatosensation – Pain

Support: R21NS120582
R21DA046184

Title: Cholinergic modulation of the descending pain circuitry

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Abstract: Acetylcholine and its receptors modulate neuronal activity to enable organisms adapt to various evolutionarily conserved and ethologically relevant stimuli. Although pharmacological agents targeting various cholinergic receptors modulate pain signaling, the impact of endogenous cholinergic circuits in acute and chronic pain conditions has not been investigated. We examined acute pain relief by cholinergic inputs to the descending pain pathway at the level of the ventrolateral periaqueductal gray (vlPAG) in adult male and female mice, and have identified pedunculopontine tegmental nucleus (PPTg) as a key mediator of cholinergic analgesia. Under chronic pain conditions, selectively stimulating cholinergic projections from PPTg to vlPAG relieved somatic and affective pain symptoms. Interestingly, chronic pain conditions are associated with decreased cholinergic tone in vlPAG, as assayed with the fluorescent ACh sensor - ACh3.0. By combining optical stimulation and pharmacology, we identified α7 nAChRs as the key cholinergic receptor mediating these analgesic effects. Finally, in-vivo calcium imaging using GCaMP6 revealed maladaptive hyper-excitability of α7 nAChR-expressing vlPAG neurons under chronic painful conditions, which is reversed by systemic treatment of α7 nAChRs agonists. These results implicate cholinergic mechanisms in acute and chronic pain-induced alterations in neuronal excitability, and will help identify novel targets for analgesia.

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Poster

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Topic: D.03. Somatosensation – Pain

Support: NIH Grant R01AT009716

Title: Insights into supraspinal mechanisms of green light-induced antinociception.

Authors: *K. CHENG[1], L. F. MARTIN[2], M. S. DENTON[2], S. M. WASHINGTON[2], T. KRANZ[2], A. M. PATWARDHAN[2], M. M. IBRAHIM[2];
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Abstract: The pain-relieving effects of green light therapy (GLED) have been characterized in animal and clinical studies, and its efficacy has been confirmed by independent groups for the treatment of a variety of pain conditions, including fibromyalgia and migraine. However, the exact underlying mechanisms utilized by GLED remain to be uncovered. Initial animal studies identified the visual system as the point of entry for GLED, though the specific photosensitive cells responsible have not yet been identified. Accordingly, hypotheses identifying either retinal cones or non-image-forming photosensitive cells as the photoreceptors responsible have arisen. Recently, a patient with protanomaly was recruited into the clinical GLED study for migraine pain. After undergoing 2 hours of GLED exposure per day for 10-weeks, the patient reported significant improvements in headache pain intensity, quality of life, and perceived headache frequency, measured by validated pain surveys. The fact that GLED was efficacious in a patient with a retinal cone pathology provides substrate for the hypothesis that intrinsically photosensitive retinal ganglion cells (ipRGCs) are key for players in GLED photoreception for pain modulation. Furthermore, previous studies suggest that GLED photoreception by the visual system ultimately acts on central pain modulation areas and the endogenous opioid system to elicit antinociception. Using pharmacological, behavioral, and in situ hybridization approaches in rodent studies, we identified that opioid receptor agonism by endogenous opioids in the rostral ventromedial medulla (RVM) was required for GLED-induced antinociception. Specifically, stereotaxic injection of naltrexone into the RVM of male rats exposed to GLED for 6 days, 8 hours/day reversed the effect of GLED in the HIV-gp120 model of neuropathic pain. Importantly, naltrexone injections only blocked mechanical, but not thermal, antinociception. In conjunction with our previous studies, these results suggest that GLED reduces descending RVM GABAergic inputs on spinal enkephalinergic interneurons to cause mechanical antinociception. Altogether, these recent findings further contribute to our understanding of supraspinal mechanisms of GLED-induced antinociception and provide the framework for future studies unveiling the exact pathway from photoreception to pain relief. An improved understanding of the mechanism of GLED antinociception will offer insights into additional clinical utility of this novel, efficacious, and non-pharmacologic pain therapy.

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Poster

P386. Descending Modulation in Pain: Brainstem and Cortex

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Program #/Poster #: P386.06

Topic: D.03. Somatosensation – Pain

Support: CIHR Foundation Grant 154281

Title: The neurochemical basis of the "anti-diffuse noxious inhibitory control" phenomenon

Authors: *W. NEMOTO[1], S. N. TANSLEY[1], J. S. MOGIL[1,2];
[1]Dept. of Psychology, [2]Alan Edwards Ctr. for Res. on Pain, McGill Univ., Montreal, QC, Canada

Abstract: Diffuse noxious inhibitory control (DNIC), alternatively known as conditioned pain modulation (CPM) or descending control of nociception, is a "counterirritation"-like phenomenon whereby pain in one location inhibits pain in another location. CPM in humans is now widely investigated as a proxy measure of endogenous pain inhibitory capacity. We recently showed that noxious conditioning stimuli can produce hyperalgesia instead of hypoalgesia depending on the intensity of the noxious test stimulus (Tansley et al., *Pain*, 2019), but the mechanism underlying our "anti-DNIC" observation is unknown. DNIC has been reported to reduce firing rates of wide dynamic range neurons, which are modulated by brainstem noradrenergic and serotonergic projection neurons, in electrophysiological studies. In this study we examined the potential involvement of serotonergic and noradrenergic neurons in the anti-DNIC phenomenon. To produce anti-DNIC, following baseline thermal pain testing using the radiant heat paw-withdrawal test (20% maximum intensity), 0.9% acetic acid was injected intraperitoneally to CD-1 mice and subsequent thermal hyperalgesia at the hind paw was measured for 30 min. Citalopram and fluoxetine, selective serotonin reuptake inhibitors, or reboxetine, a norepinephrine reuptake inhibitor, was injected s.c. or i.t. before the injection of acetic acid. The systemic administration of citalopram (30 mg/kg, s.c.) and reboxetine (30 mg/kg, s.c.) did not affect the hyperalgesia. On the other hand, this hyperalgesia was significantly attenuated by an intrathecal (i.t.) administration of reboxetine (25 µg) and fluoxetine (25 µg) 10 min before the injection of acetic acid, but it was not affected by citalopram (25 µg). These results suggest that noradrenergic and serotonergic signals in the spinal cord are involved in the anti-DNIC phenomenon. Ongoing experiments with subtype-selective antagonists will clarify the neurochemical mediation of both anti-DNIC and DNIC in awake, behaving mice.

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Poster

P386. Descending Modulation in Pain: Brainstem and Cortex

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Topic: D.03. Somatosensation – Pain

Support: NIH Grant to B.P. R01NS112194

Title: Role of Medial Prefrontal Cortex Endocannabinoids Signaling in Descending Control of Neuropathic Pain

Authors: *C. M. MECCA, Y. FENG, M. PLASKI, Q. H. HOGAN, B. PAN;
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Abstract: Medial prefrontal cortex (mPFC) is involved in pain processing, especially in engagement of descending control of pain state. In our previous study, we found that there is dynamic change of neuronal activity of mPFC after painful peripheral nerve injury (spared nerve injury, SNI), which is correlated to the dynamic change of endocannabinoid (eCB) signaling. As descending inhibitory control of pain is directly relevant to mPFC neuronal activity, we hypothesize that eCB-dependent regulation of mPFC activity after injury contributes to the descending inhibitory control of neuropathic pain. AM4113, neutral antagonist of cannabinoid receptor type 1, was microinjected into mPFC on the day of SNI and continued for 21 days to counteract the activated eCB signaling in the early phase of SNI. Sensory tests were performed to assess pain-like responses over time (before injury, 7, 14, 21, 28, 35, and 43 days after injury). SNI reliably induced hyperalgesia and allodynia, which was reversed by mPFC injection of AM4113 starting from 14 days after SNI. To understand whether analgesic effect of mPFC administration of AM4113 is by regulating descending pain modulation system, recordings from dorsal horn wide dynamic range (WDR) neuron were performed. SNI reduced mechanical threshold to induce action potential firing of WDR neurons, which was reversed in rats with mPFC injection of AM4113. These results suggested that eCB-dependent mPFC activity contributes to the pain chronification and manipulation of eCB signaling in mPFC after painful nerve injury can reverse the pain-like behaviors by regulating descending pain control system.

Disclosures: C.M. Mecca: None. **Y. Feng:** None. **M. Plaski:** None. **Q.H. Hogan:** None. **B. Pan:** None.

Poster

P394. Pain Models: Physiology

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Program #/Poster #: P394.01

Topic: D.03. Somatosensation – Pain

Support: NS098826

Title: A new tool in PAR research: the PAR2-tdTomato tagged mouse

Authors: *M. KUME[1], S. BOITANO[3], J. VAGNER[4], G. DUSSOR[2], T. J. PRICE[2];
[1]UT Dallas Cognition and Neurosci. Grad. Program, [2]Sch. of Behavioral and Brain Sci., The Univ. of Texas at Dallas, Richardson, TX; [3]Arizona Resp. Ctr. and Dept. of Physiol., [4]The BIO5 Collaborative Res. Inst., Univ. of Arizona, Tucson, AZ

Abstract: Protease-activated receptor 2 (PAR2) is a G-protein coupled receptor linked to numerous pathologies, including acute and chronic pain. Its irreversible activation by endogenous or exogenous serine-proteases is known to cause neuronal plasticity and chronic pain via the MAPK/ERK pathways. Our previous work has shown that the neurons responsible for the mechanical hypersensitivity and spontaneous pain caused by PAR2-activating proteases is a very small subpopulation of dorsal root ganglion (DRG) neurons. Despite the known therapeutic potential of targeting PAR2, progress towards a PAR2 blocking drug has been slow, partly due to the lack of PAR2-specific pharmacological tools and nonspecific antibodies. In order to address these issues, we are actively developing potent and specific PAR2 agonists and antagonists, and we have now developed a PAR2-reporter mouse where PAR2 is tagged with tdTomato on the intracellular C-terminal. This tool will potentially allow us to analyze native PAR2 expression in nociceptors and the tissues they innervate without the use of PAR2 antibodies. In preliminary experiments, we have validated PAR2-tdTomato expression in colon goblet cells and in airway epithelial cells. We have also detected PAR2-tdTomato expression in cultured trigeminal ganglion and dorsal root ganglion neurons. The PAR2-tdTomato mouse will allow us to endogenously study changes in real-time subcellular distribution of PAR2 expression, trafficking, and recycling in response to proteases, guiding the future development of PAR2 therapeutics.

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Poster

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Topic: D.03. Somatosensation – Pain

Support: NIH Grant P20GM103643

Title: Knee joint pathology and pain in mid-stage and advanced OA

Authors: T. VESEY[1], C. WILLIAMS[1], V. E. EATON[2], ***T. KING**[1];
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Abstract: Osteoarthritis (OA) is characterized by progressive articular cartilage loss, new bone formation, and synovial proliferation resulting in pain, knee joint damage, and a lower quality of life. Patients with osteoarthritis can develop different pain phenotypes. Many patients report pain during joint use that abates during rest and that can be managed with non-steroidal anti-inflammatory agents (NSAIDs), described as mid-stage osteoarthritis. Some patients report development of persistent ongoing pain that is resistant to NSAIDs, termed advanced OA. We examined potential changes in the OA joint that may contribute to differences in these pain states in a mouse model of osteoarthritis. We tested the hypotheses that mid-stage and advanced OA pain have different levels of joint pathology and pathological sprouting of sensory fibers. Notably, females develop advanced OA pain at lower MIA concentrations compared to males. We examined the hypothesis that the sex differences in pain phenotype is independent of joint pathology suggesting central mechanisms mediating these sex differences. Mice were injected with different doses of monosodium iodoacetate (MIA) that produce different pain phenotypes corresponding to mid-stage and advanced OA pain. When looking at innervation within the knee joint, there is a noticeable difference in intensities between the control, mid stage, and advanced osteoarthritis animals. Further imaging shows that in cases of advanced osteoarthritis, nerves appear to sprout into the tibialis anterior muscle. Supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences (NIGMS) of the National Institutes of Health (NIH). Histology and Imaging done in the COBRE Histology and Imaging Core supported by P20GM103643.

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Poster

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Topic: D.03. Somatosensation – Pain

Support: NIH Grant P20GM103643

Title: Changes in $GABA_A$ receptor function is dependent on osteoarthritis pain state

Authors: *V. EATON[1], K. TOWNSEND[1], T. E. KING[2];
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Abstract: GABA signaling within the spinal cord is normally associated with pain inhibition. However, changes in GABA receptor function resulting in spinal disinhibition is proposed to be integral to the maintenance of chronic pain. A murine model of monosodium iodoacetate (MIA)-induced knee joint osteoarthritis (OA) was used to investigate the hypothesis that changes in spinal GABA signaling is dependent on the characteristics of the chronic pain state. We

compared the effects of $GABA_A$ receptor activation and blockade across different OA pain phenotypes. Mid-stage OA is associated with weight asymmetry without a persistent ongoing pain state. Advanced OA is characterized by both weight asymmetry and persistent ongoing pain. Spinal administration of the $GABA_A$ agonist (muscimol) and antagonist (bicuculline) were used to investigate alterations in $GABA_A$ receptor signaling across mid-stage and advanced-stage OA pain states. Our experiments demonstrate that spinal bicuculline did not alter weight asymmetry in mice with mid-stage OA whereas it normalized weight bearing in mice with advanced OA. Spinal muscimol normalized weight asymmetry in mice with mid-stage OA. Conversely, advanced OA mice showed no change in weight bearing. In mice with advanced OA, muscimol induced conditioned place aversion, indicated worsening of joint pain, whereas bicuculline induced conditioned place preference, indicating that bicuculline produced pain relief. Additionally, optogenetic activation of GABAergic interneurons showed normalization of weight bearing in mid-stage but not advanced OA mice, further indicating a paradoxical switch in spinal GABA function is only observed in a chronic OA pain state associated with persistent ongoing pain. Potassium chloride channel 2 (KCC2) has been implicated as a key mechanism driving altered $GABA_A$ receptor function in chronic pain. Restoring KCC2 function through spinal administration of CLP290 reversed weight asymmetry in mice with advanced but not mid-stage OA. These data indicate that $GABA_A$ receptors induce net inhibition in mid-stage OA, a chronic pain state characterized by intermittent joint pain. In contrast, $GABA_A$ receptors induce net excitation in advanced OA, a chronic pain state characterized by persistent ongoing joint pain. Supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences (NIGMS) of the National Institutes of Health (NIH, P20GM103643).

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Poster

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Topic: D.03. Somatosensation – Pain

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Title: Analgesic Dorsal Root Ganglionic Field Stimulation Blocks Conduction of Afferent Impulses in Rats with Monoiodoacetate-induced Osteoarthritis

Authors: D. CHAO, Q. HOGAN, *B. PAN;
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Abstract: Chronic joint pain is common in patients with osteoarthritis (OA). Non-steroidal anti-inflammatory drugs and opioids were used to relieve OA pain and their effects are not satisfied.

Dorsal root ganglion field stimulation (GFS) is an analgesic neuromodulation approach in use clinically. Even though it is not currently used in patients with OA pain, GFS is effective in a preclinical rat OA pain model. Here, we evaluated the cellular mechanism of analgesic effects of GFS on OA pain with electrophysiological teased fiber recordings in a monosodium iodoacetate (MIA)-induced OA (MIA-OA) rat model. Two weeks after intra-articular injection of MIA, those rats developed hypersensitivity to knee press stimulation, spontaneous pain with incapacitance test, and hypersensitivity to punctate mechanical stimulation to plantar skin of hind paw. In rats with MIA-OA, *in vivo* single unit recordings from nerve bundles teased from the saphenous nerve and third lumbar (L3) dorsal root showed higher incidence of spontaneous activities (SA) than those from control rats. GFS at L3 level blocked this SA. After MIA-OA, the threshold to induce action potential (AP) firing by punctate mechanical stimulation (von Frey) was reduced when recorded from bundles teased from the L4 dorsal root, which was reversed to normal after GFS. These results indicate that GFS takes analgesic effects in MIA-OA rat model by producing use-dependent blockade of SA and afferent AP trains induced by either knee moving or punctate mechanical stimulation to paw.

Disclosures: D. Chao: None. **Q. Hogan:** None. **B. Pan:** None.

Poster

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Title: Evidence of autonomic dysfunction in the male rat after surgical induction of osteoarthritis

Authors: *T. YEATER[1], J. ZUBCEVIC[2], J. GRIFFITH[1], K. ALLEN[1,3];
[1]J. Crayton Pruitt Family Dept. of Biomed. Engin., [2]Dept. of Physiological Sci., [3]Dept. of Orthopedic Surgery and Sports Med., Univ. of Florida, Gainesville, FL

Abstract: *Objective:* Functional changes in the autonomic nervous system may help explain variability in the progression of knee osteoarthritis (OA). Indeed, autonomic dysregulation is implicated in rheumatoid arthritis, another chronic inflammatory joint disease. In this study,

autonomic nervous system shifts, as measured via heart rate response variables, were evaluated in rat knee OA models. *Methods:* Radiotelemetry probes were implanted to collect blood pressure measurements from which the heart rate was derived from the peaks in pulse pressure. Heart rate was evaluated as an indirect assessment of vagal nerve function, a key part of the autonomic nervous system. Recordings occurred at baseline and every other week for 8 weeks after induction of OA via medial collateral ligament transection plus medial meniscus transection (MCLT+MMT; n=7-10) or MCLT alone (n=4-5), with skin incision (n=7-9) used as a control. Cardiovascular responses were assessed in response to 1-phenylbiguanide, which has reported vagal activation properties (n=5-6/group). Cardiovascular responses to mechanical stimuli applied at the knee were used to indirectly assess cardiac vagal responses. Finally, changes in cartilage and bone architecture were quantified using custom artificial intelligence driven histological analysis pipeline. Experiments were done in accordance with ARRIVE guidelines. *Results:* Cardiac vagal tone decreased in MCLT+MMT animals during high activity periods, but not low activity. However, during low activity, MCLT and MCLT+MMT animals exhibited a slower heart rate than controls ($p<0.05$, week main effect). Mechanical stimuli at the knee resulted in a sudden drop in heart rate and blood pressure, with no differences seen between groups. Additionally, MCLT and MCLT+MMT animals tended to have a larger drop in heart rate following administration of 1-phenylbiguanide ($p=0.06$, surgery*dose interaction). Histological analysis revealed cartilage loss and subchondral bone thickening in MCLT+MMT animals compared to control and MCLT animals ($p's<0.05$). *Conclusions:* Acute mechanical stimulation of the knee produced an immediate reduction in heart rate, suggesting a direct neural joint-brain connection that may modulate autonomic responses in OA. Measurement of cardiovascular responses to pharmacological stimulation of the vagus nerve via 1-phenylbiguanide, which is reportedly able to induce acute cardiac vagal responses, demonstrate for the first time a shift in autonomic function in rodent knee injury models of OA. This, combined with the more prominent chronic reduction in heart rate in OA groups, suggests sensitization of cardiac vagal activation with OA.

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Poster

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Jefferson Synaptic Biology Center

Title: Neuropod-based modulation of DRG neuron excitability may underlie the pathophysiology of visceral abdominal pain

Authors: *T. D. ALEXANDER[1], J. BARTON[2], S. WALDMAN[2], M. COVARRUBIAS[1]; [1]Dept. of Neurosci. and Vickie and Jack Farber Inst. for Neurosci., [2]Dept. of Pharmacol. and Exptl. Therapeut., Thomas Jefferson Univ., Philadelphia, PA

Abstract: Visceral abdominal pain affects around 10% of the US population and is a major burden on the healthcare system – accounting for over $40 billion in medical costs. The mechanisms underlying visceral abdominal pain are complex and involve spinal cord afferent neurons, including dorsal root ganglia (DRG) neurons, which signal to higher order pain centers. It has been recently shown that a new type of intestinal epithelial cells known as neuropods exhibit neuron-like properties and may synapse on spinal cord afferents. These neuropods are receptive to nociceptive cues and may communicate with visceral afferents through specialized chemical synapses. However, the underlying electrical properties of the neuropod and the spinal cord afferents with which they communicate have yet to be understood. Exploring this unique epithelial-neural signaling axis provides insight into the pathophysiology of visceral pain and reveals neuropod cells as a potential therapeutic target. To examine the underlying electrical properties of this system, we performed patch-clamp recordings on both DRG neurons and neuropods in coculture. Mouse neuropods expressing a fluorescent reporter and the surrounding crypt cells from small intestine were cocultured with mouse spinal cord embryonic DRG neurons, and various recording protocols were used to examine underlying electrical properties. Neuropods exhibited voltage-dependent inward and outward currents and a resting membrane potential (RMP) of -62 mV (range -27 to -95 mV, n=2); however, upon sustained current injection up to 180 pA for 1 s, neuropods did not fire action potentials (APs). Insufficient Na conductance under our experimental conditions may underlie this property. The RMP of DRG neurons with processes extending towards neuropods was comparable to that of DRG neurons not extending towards the neuropods (-60±11 mV, n=8, and -67±19 mV, n = 4, respectively, P=0.29). Remarkably, upon sustained current injection of 60 pA for 1 s, DRG neurons extending towards neuropods fired a median of 10 APs while DRG neurons not extending towards neuropods fired a median of 1 AP (P=0.01). DRG neurons which interact with neuropods may be hypersensitive, potentially as a result of signaling between these two cells. An increase in this signaling may manifest as increased visceral abdominal pain. We are currently examining the neuropod-specific effects of these changes, further effects on AP dynamics of both the neuropod and DRG neurons, and the implications in visceral abdominal pain.

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Poster

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Title: Acid-sensing ion channels modulate bladder nociception

Authors: N. MONTALBETTI, ***M. D. CARATTINO**;
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Abstract: Acid-sensing ion channels (ASICs) are cation permeable channels expressed in neurons that respond to extracellular acidification. In this report, we investigated the contribution of ASICs to bladder nociception in a model of chemical-induced cystitis. Female and male mice were injected intraperitoneally with a cyclophosphamide (CYP) solution (80 mg/kg) every other day for up to a week. We found that the administration of CYP to both female and male mice lacking ASIC3, a subunit primarily expressed in sensory neurons, generates pelvic allodynia at a time point at which only modest changes in pelvic sensitivity are apparent in wild type (WT) mice. To assess whether the referred pelvic allodynia seen in *Asic3* KO mice treated with CYP is caused by sensitized primary afferents, we examined the firing evoked by sustained electrical stimulation of acutely isolated bladder sensory neurons from WT and *Asic3* KO mice. Sensory neurons were classified based of the sensitivity of the action potential to tetrodotoxin (TTX), as TTX-resistant (TTX-R) or TTX-sensitive (TTX-S). As expected, sensory neurons from both WT and *Asic3* KO mice treated with saline display a phasic pattern of action potential firing in response to suprathreshold stimulation. In contrast, and consistent with sensitization, we noticed that sensory neurons with TTX-S action potentials from both groups of mice treated with CYP exhibited tonic firing. But strikingly, CYP treatment only sensitized neurons with TTX-R action potentials in *Asic3* KO mice. These findings indicate that the differences in mechanical pelvic sensitivity observed between WT and *Asic3* KO mice treated with CYP are mediated by sensitized bladder nociceptors with TTX-R action potentials. Lastly, our studies showed that the loss of ASIC3 from sensory neurons abolished their ability to discharge action potentials in response to extracellular acidification. Collectively, our studies support the notion that protons and their cognate ASIC receptors are part of a mechanism that operates at the nerve terminals to modulate nociceptor excitability and sensitization.

Disclosures: N. Montalbetti: None. **M.D. Carattino:** None.

Poster

P394. Pain Models: Physiology

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P394.08

Topic: D.03. Somatosensation – Pain

Support: NIH NS045594
NIH HEAL Supplement to NS045594

Title: TGF-β/P2X4 signaling pathway in sympathetic-mediated inflammatory low back pain

Authors: D. D. LÜCKEMEYER[1], W. XIE[1], R. TONELLO[2], T. BERTA[1], *J. A. STRONG[1], J.-M. ZHANG[1];
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Abstract: Although the sympathetic nervous system drives several pain conditions and local sympathetic blockade or lesion is clinically used to treat pain, the underlying mechanisms are remain poorly understood. To better understand these mechanisms, we developed in rats and mice a localized microsympathectomy (mSYMPX), which consists of cutting the grey rami containing postganglionic sympathetic fibers as they enter the spinal nerves near the lumbar dorsal root ganglia (DRG). We reported that this mSYMPX attenuated pain behaviors in several preclinical pain models including a mouse model of chemotherapy-induced peripheral neuropathy (CIPN model). Attenuation of pain behavior by mSYMPX in the CIPN model involved macrophages and anti-inflammatory actions of transforming growth factor-β (TGF-β) and its receptor TGF-βR1 at the level of the DRGs. In this study we asked whether the macrophage-enriched purinergic receptor P2RX4 and anti-inflammatory cytokine TGF-β are involved in sympathetic-mediated inflammatory low back pain/radiculopathy produced by intravertebral injection of the immune stimulator zymosan, causing mechanical hypersensitivity (LID model). Our previous microarray study in rats showed regulation of the macrophage cell marker ionized calcium binding adapter molecule 1 (*Iba1*), *P2rx4*, *Tgfb1* and its receptor *Tgfbr1* after LID. As previously reported in rats, we report that mice showed mechanical hypersensitivity assessed by calibrated von Frey filaments and transcriptional upregulation of *Iba1*, *Tgfb1* and *Tgfbr1* three days after LID. Interestingly, mSYMPX prevents mechanical hypersensitivity and it is associated with significant transcriptional upregulation of *Iba1*, *Tgfb1* and *Tgfbr1* and potential downregulation of P2rx4 (p=0.1) three days after LID. These findings suggest TGF-β signaling as a general underlying mechanism of local sympathetic blockade or lesion in several pain conditions, and indicate TGF-β and potentially P2RX4 as therapeutic targets to treat inflammatory low back pain/radiculopathy.

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Poster

P394. Pain Models: Physiology

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Program #/Poster #: P394.09

Topic: D.03. Somatosensation – Pain

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Title: Human TrkA^{R649W} and human NGF^{R100W} mutations impair nociception but differentially affect sweating and cognitive abilities: two mouse models of HSAN IV and V diseases

Authors: *P. PACIFICO[1], G. TESTA[1], R. AMODEO[2,1], L. MARCHETTI[3,4], J. ARÉVALO[5], S. CAPSONI[1,6], A. CATTANEO[1,7];
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Abstract: In adulthood, the neurotrophin Nerve Growth Factor (NGF) produces a long-lasting thermal and mechanical hyperalgesia after tissue damage by activating its receptors TrkA and P75^{NTR}. The necessity for a functioning NGF-TrkA system is evident in individuals affected by Hereditary Sensory and Autonomic Neuropathies type IV and V (HSAN IV and V), caused by mutations in TrkA or NGF, respectively. Both diseases are characterized by loss of response to noxious stimuli while anhidrosis and variable mental retardation are found exclusively in HSAN IV patients. However, how mutations in TrkA or in NGF can lead to different clinical phenotypes remains to be discovered. To this aim, our laboratory previously generated a knock-in mouse line harboring the HSAN V R100W mutation in the human NGF gene (NGF^{R100W}). In agreement with HSAN V patients, NGF$^{R100W/m}$ mice lack to respond to noxious stimuli preserving cognitive abilities (Testa et al., 2019). Here, we show that the R649W mutation in TrkA tyrosine kinase domain affects phosphorylation in response to the binding of NGF without modifying the total amount of the protein. We found that R649W mutation decreased the ubiquitination and the membrane mobility, leading to an increase of the density of TrkA^{R649W} at the cell membrane surface. To verify whether TrkA^{R649W} influences *in vivo* the physiology of dorsal root ganglia (DRG) sensory neurons and pain sensitivity, we generated a knock-in mouse carrying the mutation R649W in the human TrkA. In the homozygous condition, the TrkA^{R649W} mutation is lethal, as observed in NGF$^{R100W/R100W}$ mice (Testa et al, 2019). We also demonstrated that, in both male and female heterozygous TrkA^{R649W} mice, nociception is specifically impaired, with a reduction of the response to thermal- and chemical- noxious stimuli. Moreover, we found that TrkA^{R649W} affects the expression of nociceptive markers in DRG and the skin innervation. Using a sweat assay, we observed that anhidrosis is strictly related to TrkA^{R649W} while no sweating deficits are detected in NGF$^{R100W/m}$. Finally, we showed that cognitive abilities linked to spatial-working memory, anxiety and sociability are impaired only in TrkA$^{R649W/m}$ mice. We conclude that not only TrkA$^{R649W/m}$ and NGF$^{R100W/m}$ mice accurately reproduce the clinical manifestations of the respective pathologies but suggest unexplored novel roles of TrkA in

thermoregulation and in sociability. The comparison between these transgenic mice could provide new knowledge about the role of NGF-TrkA signaling in nociception and pain perception, possibly leading to the isolation of new candidates for the development of innovative therapeutic strategies to alleviate chronic pain.

Disclosures: P. Pacifico: None. **G. Testa:** None. **R. Amodeo:** None. **L. Marchetti:** None. **J. Arévalo:** None. **S. Capsoni:** None. **A. Cattaneo:** None.

Poster

P420. Olfactory Coding: Olfactory Bulb II

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P420.01

Topic: D.05. Olfaction and Taste

Support: NIH Grant DC014701
NIH Grant DC014367
NIH Grant DC017382

Title: Dynamics of spike time encoding in the olfactory bulb

Authors: *J. C. WERTH, T. A. CLELAND;
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Abstract: Synchronized oscillatory activity at gamma frequencies (30-100 Hz) occurs in a variety of brain circuits which process sensory and perceptual information. In the olfactory bulb, the odor-evoked activity of principal neurons (mitral and tufted cells; MTCs) shows striking periodicity and synchronization, which is known to be necessary for fine odor discrimination in the analogous insect antennal lobe. While the relevance of spike timing on fast gamma timescales is clear, we lack a full understanding of how sensory information is embedded in this temporal framework - in part due to a limited capacity to control the receptor activation pattern, intensity profile, and timecourse of odor delivery on fine spatial and temporal scales.
We investigated the spike-timing properties of MTCs using an in vitro bulbar slice preparation mounted on a 120-channel planar multielectrode array, while delivering “artificial odor” stimuli using spatiotemporally patterned optogenetic stimulation. These “artificial odor” stimuli evoked persistent gamma oscillations in the local field potential, and generated gamma-synchronized spiking (lag $\leq$ 5 ms) among recorded MTCs. This synchrony occurred in an “odor”-specific manner, such that the MTCs with synchronized spikes depended on which “artificial odor” was presented, and were largely conserved across multiple presentations of the same “artificial odor”. These results suggest that MTC spike timing properties are conserved in OB stimulus representations.

Disclosures: J.C. Werth: None. **T.A. Cleland:** None.

Poster

P420. Olfactory Coding: Olfactory Bulb II

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Program #/Poster #: P420.02

Topic: D.05. Olfaction and Taste

Support: K99DC019505
UF1NS111692
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T32NS043124

Title: Active olfactory sensing and state-dependent regulation of olfactory processing by the basal forebrain

Authors: *E. HANSON MOSS[1], C. SMITH[2], P. J. PFAFFINGER[3], J. REIMER[3], B. R. ARENKIEL[4];
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Abstract: Top-down regulation allows flexible modulation of sensory processing in response to behavioral states like attention and arousal.Understanding the impact of state-dependent modulation on olfactory processing has been limited by the spatial scale and temporal resolution of in vivo imaging platforms. Leveraging high-speed, meso-scale two-photon imaging in mice, we examine how spontaneous shifts in attention and arousal modulate spatiotemporal properties of odor responses across the olfactory bulb. Here, we describe an approach combining mesoscopic imaging of the olfactory bulb with temporally precise odor delivery and detailed behavioral state monitoring in awake mice. Using this approach, we visualize olfactory bulb odor response dynamics across spontaneous changes in behavioral state and with manipulations of state-dependent basal forebrain neuronal activity. To quantify active olfactory sensing, we apply a novel method using video-based tracking of nostril motion and temperature-based respiration recording. Additionally, we relate active olfactory sensing states to measures of arousal including wheel running and eye area to create a more comprehensive measure of behavioral state changes over time and across odor presentations. Then, using simultaneous, meso-scale two-photon recordings of olfactory bulb mitral cell somas and glomeruli, we relate glomerular and mitral cell body activity to active sensing and arousal states during passive odor exposures. Finally, using chemogenetic inhibition, we demonstrate how cholinergic input from the basal forebrain impacts state-dependent and odor-evoked olfactory bulb activity. Ultimately, our approach for combined behavioral state monitoring and mesoscopic two-photon imaging allows us to investigate how spontaneous behavioral state changes in awake mice modulate odor responses and

spatiotemporal patterns of activity across the glomerular and mitral cell layers of the olfactory bulb.

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Poster

P420. Olfactory Coding: Olfactory Bulb II

Location: Virtual Only

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Program #/Poster #: P420.03

Topic: D.05. Olfaction and Taste

Support: NIH Grant DC016133
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Title: The impact of odor stimulation on the responsiveness of mitral/tufted cell glomeruli.

Authors: N. SUBRAMANIAN, ***D. A. STORACE**;
Biol. Sci., Florida State Univ., Tallahassee, FL

Abstract: Localizing an odor requires that an organism carry out several perceptual computations in parallel. An odor percept of interest must be recognized at some distance from the source despite the presence of other background stimuli and approaching (or avoiding) the stimulus requires maintaining both sensitivity to concentration changes and a concentration-invariant representation of the odor. Here we examined whether mitral/tufted cells in the olfactory bulb could be involved in these perceptual functions. Odor-evoked activity was measured from mitral/tufted cell apical dendrites in the olfactory bulb glomerular layer using 2-photon calcium imaging in awake mice in response to a large range of concentrations presented with varying interstimulus intervals. Different mitral/tufted cell glomeruli exhibited a range of concentration sensitivities including those that changed monotonically over the tested concentration range, and others that were relatively concentration invariant. Subsets of MTC glomeruli also exhibited significant adaptation when odors were delivered repeatedly. The results suggest that different subsets of MTC glomeruli may be involved in coding for concentration changes, odor identity, and background segmentation.

Disclosures: N. Subramanian: None. **D.A. Storace:** None.

Poster

P420. Olfactory Coding: Olfactory Bulb II

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Program #/Poster #: P420.04

Topic: D.05. Olfaction and Taste

Support: DGAPA-UNAM AG200521
CONACYT A1-S-7540
Fellowship CONACYT

Title: Main olfactory bulb reconfiguration by prolonged passive experience correlates with increased BDNF and improved innate olfaction.

Authors: *R. HERNÁNDEZ-SOTO[1], A. PIMENTEL-FARFAN[2], E. ADAN-CASTRO[2], C. CLAPP[2], F. PEÑA ORTEGA[2];
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Abstract: The main olfactory bulb (MOB) can reconfigure its function and structure in response to acute and chronic environmental changes. Despite the extensive evidence of anatomical, functional, and behavioral changes in the olfactory system induced by a wealth of olfactory exposure protocols, it is still unknown whether prolonged passive odor experience could reconfigure the MOB at its input and network activity levels and whether these changes impact innate olfaction. To test these possibilities, we passively exposed adult CD-1 mice to 21 different odors for 21 days to later record their MOB glomeruli activation, with intrinsic optical signals, and their MOB local field potential in the anesthetized condition. Furthermore, we analyzed their olfactory innate detection and discrimination with the habituation/dishabituation test and characterized their preference/avoidance to attractive and aversive odors, respectively. Our results showed that mice chronically exposed to odors exhibit a reconfiguration in their glomerular maps that includes a reduction in the number of activated glomeruli, an increase in their activated area, and an enhancement in their intrinsic signals. We also observed a reconfiguration in the MOB population activity characterized by a decrease in slow oscillations (<12 Hz) and an increase in fast oscillations (>12 Hz), both in the absence and the presence of odors. Mice chronically exposed to odors also increased their sensibility to olfactory stimuli during the habituation/dishabituation test and improved their innate odor preference/avoidance. These changes in olfaction and the reconfiguration of the MOB function correlated with an increase in local BDNF (brain-derived neurotrophic factor) levels. Our results showed that prolonged olfactory experience reconfigures the MOB in adults, probably through BDNF-dependent mechanisms, and improves innate olfaction.

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Poster

P420. Olfactory Coding: Olfactory Bulb II

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Program #/Poster #: P420.05

Topic: D.05. Olfaction and Taste

Support: NIH Grant R01 DC016560

Title: Birthdate-dependent differences in the functional connectivity of postnatal-born olfactory granule cells

Authors: *J. S. HUANG[1], T. R. BRECHBILL[1], A. LAUER[1], N. G. GLASGOW[1], C. E. J. CHEETHAM[1], N. N. URBAN[2];
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Abstract: Throughout life, the rodent olfactory bulb (OB) is continually supplied with new postnatal-born neurons originating as neural stem cells from the subventricular zone. About 90% of these postnatal-born neurons differentiate into olfactory bulb granule cells (GCs). GCs mediate lateral inhibition via synapses with mitral cells (MCs) and tufted cells (TCs), the primary output neurons of the olfactory bulb (OB). Anatomical evidence indicates that early postnatal-born GCs (P3-P7) occupy the superficial GCL, whereas late postnatal-born GCs (P14-60) are located in the deeper GCL (Lemasson et al. 2005). Like the dendrites of MCs and TCs, the superficial and deep subpopulations of GCs also extend their dendrites to distinct depths of the external plexiform layer (EPL) (Mori et al. 1983, Orona et al. 1983). It is thus plausible that GCs make preferential connections with MCs and TCs depending on their postnatal birthdate. In this study, we aimed to determine whether anatomical differences in OB circuitry translate to functional differences in connectivity. We used timed viral injections, optogenetics, and slice electrophysiology to test whether early postnatal-born GCs are preferentially connected with TCs, and whether late postnatal-born GCs are functionally connected with both MCs and TCs. We injected AAV2 Syn1:ChR2-YFP virus into the RMS of P2 and P14 C57BL/6NCrl mice to express YFP-tagged ChR2 in either early (P2) or late (P14) postnatal-born GCs. We sacrificed both cohorts for experiments at 4 wpi and recorded IPSCs in MCs and TCs following optogenetic stimulation of virally transduced postnatal-born GCs. Optogenetic activation of P2-transduced GCs resulted in IPSCs in 47% of MCs (n = 15 cells) and 42% of TCs (n = 12 cells). In contrast, optogenetic activation of P14-transduced IPSCs resulted in IPSCs in 81% of MCs (n = 21 cells) and 40% of TCs (n = 15 cells). At both timepoints, MCs showed larger light-evoked IPSC amplitudes than TCs (-22.7 pA vs. -7.7 pA for P2, and -185.0 pA vs. -36.2 pA for P14; unpaired t-tests, $p < 0.05$ and $p < 0.02$). These findings suggest that both early and late postnatal-born GCs provide stronger inhibition onto MCs than onto TCs. Activation of early postnatal-born GCs evoked IPSCs in a similar proportion of MCs and TCs, suggesting a similar level of connectivity between MCs and TCs and early postnatal-born GCs. Late postnatal-born GCs, however, seem to preferentially connect with MCs over TCs. These results provide evidence for

differences in functional connectivity between subpopulations of postnatal-born GCs and olfactory bulb output neurons.

Disclosures: J.S. Huang: None. **T.R. Brechbill:** None. **A. Lauer:** None. **N.G. Glasgow:** None. **C.E.J. Cheetham:** None. **N.N. Urban:** None.

Poster

P420. Olfactory Coding: Olfactory Bulb II

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Program #/Poster #: P420.06

Topic: D.05. Olfaction and Taste

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Title: Odor information processing by glomerular interneurons of the olfactory bulb : a structural/functional in vivo imaging study

Authors: *M. LOIZEAU[1], A. ANGELOVA[2], B. DETAILLEUR[1], H. CREMER[1], H. ROUAULT[2], J.-C. PLATEL[2];
[1]IBDM, CNRS, Aix-Marseille Univ., Marseille, France; [2]Inmed, INSERM, Aix-Marseille Univ., Marseille, France

Abstract: Olfaction is a crucial sense in animals allowing them to survive and reproduce. The first step of olfactory processing takes place in olfactory bulb (OB), in specialized structures called glomeruli that receive axons from olfactory sensory neurons. In glomeruli, the olfactory signal is processed by a variety of interneurons while being transferred to OB output neurons, the mitral and tufted cells. Yet, due to the high density and diversity of interneurons, the role of each population of interneurons in the OB circuit is still fragmentary. We aim to understand how interneurons that project on the same glomerulus are activated, how they participate in the odor processing and in particular how they code odor concentration.We chose to focus on a specific population of interneurons of the glomerular layer, the external tufted cells (ETCs). ETCs, which are the only excitatory interneurons in the OB, represent an entry door to study population coding inside the glomeruli. We crossed the ND6 transgenic mouse line with a fluorescent reporter and a calcium genetic sensor line. We designed a new experimental approach by combining high resolution two-photon structural imaging to reconstruct the morphology of ETCs and assign them to a specific glomerulus, with calcium imaging to record their response to odors *in vivo*. By using this approach, we observed that upon olfactory stimulation, ETCs can be activated and surprisingly, they can also be inhibited or generate complex responses *in vivo*.

These response profiles are similar to the one reported for OB output neurons, which could suggest that ETCs drive their activity *in vivo*. We showed that the ETCs display an odor-dependent response profile: the same ETC can be activated by one odor and inhibited by another. However, ETCs attached to the same glomerulus respond with the same response profiles, reinforcing the idea that glomeruli behave as specific functional units in the OB. Then, we characterized how interneurons connected to a single glomerulus respond to increasing odor concentrations. We showed that ETCs are progressively responding in a concentration dependent manner, representing a cellular recruitment map for each glomerulus. We aim to observe if these cellular recruitment maps change or remain stable over time varying from days to months. Our work describes both the properties of ETCs in the OB circuit and a new cellular mechanism for odor concentration coding in glomeruli.

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Poster

P420. Olfactory Coding: Olfactory Bulb II

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Program #/Poster #: P420.07

Topic: D.05. Olfaction and Taste

Support: NIH/NINDS R24 NS098536

Title: Bilateral sensory signals for odor source localization in freely-moving mice

Authors: *K. A. BOLDING[1], J. TAI[1], D. P. LEMAN[2], I. G. DAVISON[1];
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Abstract: During sensory-guided navigation, animals constantly refine their ongoing movement through a series of dynamic, iterative sensory-motor algorithms. In natural contexts, odors form key navigational cues that signal food sources and social partners, offering an ethologically relevant window on motivated sensory search. While odor-evoked activity has been studied intensively in head-fixed animals and decision-making tasks, little is known about the nature of the dynamic sensory signals that guide freely moving animals during active sampling of their environment. Such data is critical to resolve the sensory-motor strategies available to the animal at each stage of their sensory search. Animals may navigate using comparison of signals across successive discrete 'sniff' samples, using instantaneous 'stereo' comparison across hemispheres, or employ both under different conditions. To overcome the challenges of measuring bilateral odor responses in unrestrained animals, we developed new miniaturized microscopy tools for large-scale visualization of neural activity, and used them to image both hemispheres of the main olfactory bulb in mice exploring odor sources in an open arena. Sensory-evoked activity was

detectable only within a restricted area of ~10 cm surrounding the odor source and occurred in discrete sensory bursts, consistent with intermittent odor encounters and active sampling. Increasing proximity to the source activated additional glomeruli, revealing that spatial information is encoded by progressive recruitment of receptor pathways of varying affinity. A bias in directional preference for stimuli nearest the corresponding naris emerged in a subset of glomeruli at close proximity. Bilateral imaging enabled the identification of homologous pairs of glomeruli with correlated signals in either hemisphere. The difference between these left and right signals produced strongly biased directional tuning and predicted a bias in turning direction in a motivated foraging task. These data suggest that animals may employ multiple strategies to localize odor sources during free exploration, initially comparing the degree of glomerular recruitment across time during early approach phases, and ultimately reading out a bilateral direction code at close proximity.

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Poster

P433. Auditory Processing: Temporal, Frequency, and Spectral Processing IV

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Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P433.01

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant DC003180

Title: Neuronal classes in primate auditory cortex have different temporal response dynamics

Authors: *X.-P. LIU[1], X. WANG[2];
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Abstract: Neurons in auditory cortex show a diversity of response properties in their spiking activity, but it is unclear to what extent this diversity results from neuronal type diversity. We collected single-unit recordings from auditory cortex of awake marmosets, and classified these units into three dominant classes based on extracellular waveform, spike timing, and spontaneous rate criteria. Most units were classified as regular spiking while a substantial minority were classified as fast spiking or bursting. A second method using unsupervised clustering on a set of informative features identified the same dominant classes with high agreement. Based on this classification, we find that the temporal dynamics of responses to synthetic and natural stimuli are strongly influenced by unit type. Among other differences, fast spiking units exhibited the weakest adaptation, while bursting units exhibited the strongest adaptation, preferentially responding at the onsets of sounds. Correspondingly, in response to sinusoidally amplitude modulated stimuli, bursting units had high vector strengths, could phase-lock to high

modulations rates, and peaked early in the rising phase of each cycle. These differences were also present in responses to conspecific vocalizations, with bursting units firing transiently at particular moments, reflected in higher values of the correlation index metric (Joris et al., 2006). Bursting units showed indication of finer spike timing information in their vocalization responses as assessed by a decoding metric spanning from rate to temporal coding (Victor and Purpura, 1996). We hypothesize that bursting units may implement a transformation accentuating acoustic edge landmarks and form the basis for parallel temporal and rate codes in auditory processing.

Disclosures: X. Liu: None. **X. Wang:** None.

Poster

P433. Auditory Processing: Temporal, Frequency, and Spectral Processing IV

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Program #/Poster #: P433.02

Topic: D.06. Auditory & Vestibular Systems

Support: NSF SMA-1734892
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Title: Network localized Granger causality reveals cortical network changes under difficult listening conditions

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Abstract: Growing evidence from neuroimaging suggests that networked activity of different cortical areas may facilitate speech comprehension under difficult listening conditions. Inferring the underlying causal relationships is critical for understanding the neural mechanisms behind successful speech comprehension. Granger causality (GC) has been widely used for this purpose in functional magnetic resonance imaging analysis, however, due to the low temporal resolution there, it is not possible to capture millisecond-scale interactions. Magnetoencephalography (MEG) provides millisecond resolution, but only gives low-dimensional, noisy, and linearly mixed surrogates of cortical activity, thus making GC inference a challenging task.
Existing methods for cortical network inference typically proceed in two stages by first performing MEG source localization, followed by GC inference. As such, the spatiotemporal biases induced by source localization in the first stage may propagate to the second stage and result in false alarms and missing the true GC links. To address this challenge, we introduce the

network localized Granger causality (NLGC) inference paradigm, in which cortical source dynamics are modeled as latent sparse multivariate autoregressive processes whose parameters are directly estimated from the MEG measurements, without the need for intermediate source localization.
We demonstrate the utility of NLGC inference using experimentally recorded MEG data from younger and older participants performing an auditory task: sustained listening to speech under different noise conditions. In analyzing the networked activity of frontal, temporal, and parietal areas, NLGC reveals novel hemispheric and lobe-specific dependence of network connectivity patterns in younger vs. older adults and across task difficulty. The age effects are more prominent in the most difficult listening conditions. Interestingly, the network connectivity patterns dissociate from the time-locked activity levels. Our results suggest that NLGC inference can be used as a robust alternative to existing methods for network-level analysis of MEG data that underlie auditory processing.

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Poster

P433. Auditory Processing: Temporal, Frequency, and Spectral Processing IV

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Program #/Poster #: P433.03

Topic: D.06. Auditory & Vestibular Systems

Support: NSF-BCS grant 1840818

Title: Association of musical training with auditory and speech neural coding and perception

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Abstract: Numerous studies have reported a link between musical training and enhanced neural processing and perception of sound, including speech in “noise”. However, the generalizability

of these effects remains unclear for several reasons, including small samples, small and inconsistent effect sizes, and varying methodological and analytical techniques. To address these issues, we are conducting a large-scale, multi-site study involving six universities, aiming to examine the robustness of the purported musician advantage across the adult lifespan by replicating and extending eight key experiments involving the perception and neural coding of speech and non-speech sounds across a large sample of listeners spanning a wide range of musical experience. All six sites collected data for all eight experiments. Preliminary data have been collected from n=173 subjects (n=104 female; n=67 male; n=2 other; ages 18-83 years old), with a final sample size goal of $n \geq 300$. Behavioral measures include melody perception, pitch discrimination, speech-in-noise perception, speech and non-speech informational masking, frequency selectivity, and extended high frequency thresholds. Neural measures include scalp-recorded EEG responses to passively attended phonemes (/da/ in multi-talker babble and the Mandarin tone /mi3/ in quiet). Behavioral trends suggest the musician advantage is most robust for tasks directly relevant to musical training (e.g., melody perception and pitch discrimination). So far, no evidence has emerged for enhanced neural encoding of sound in musicians relative to non-musicians (quantified as spectral encoding for the F0 or upper harmonics at electrode Cz). Formal statistical analyses are preregistered on the Open Science Framework (https://osf.io/duq34/) and will be conducted once full data collection is complete.

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Poster

P433. Auditory Processing: Temporal, Frequency, and Spectral Processing IV

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P433.04

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant P50MH109429
NIH Grant R01DC014279

Title: Entrainment effects to simple sounds and speech in human auditory cortex revealed by intracranial recordings

Authors: *S. AKKOL[1,2], A. MISHRA[3], N. MARKOWITZ[3], M. KESHISHIAN[4], P. LAKATOS[6], N. MESGARANI[5], A. D. MEHTA[7], S. BICKEL[8];
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Abstract: The entrainment of neural oscillations in the auditory system to acoustic inputs has been proposed as a mechanism aiding processing of simple sounds and speech. However, evoked potentials to prominent acoustic events complicate the measurement of neural oscillations. One potential way to establish the presence of entrained oscillations is to measure if they outlast acoustic stimulation. While there are multiple non-human and human studies using this approach, some show positive and others negative results potentially due to methodological differences. Here, we used both simple sounds and speech stimuli in two tasks while recording EEG invasively in epilepsy patients to elucidate if the neural entrainment outlasts the stimulus duration. We leveraged the high spatial and temporal resolution of invasive electroencephalography recordings in a total of 17 epilepsy patients (9 females). In the first experiment, 12 participants listened to 4 simple sounds presented at a rate of 2.8 Hz or 3.7 Hz and were instructed to detect a specific sound in noise presented at various offsets to the entrainment sounds. We analyzed 43 contacts in Heschl's gyrus (HG) and 24 in the superior temporal gyrus (STG) from the 2.8 Hz condition, and 56 in HG and 27 in STG contacts from the 3.7 Hz condition. We found no evidence of ongoing oscillations at stimulation frequencies outlasting entrainment stimulus presentation for more than one cycle in either locations ($F(3,438)=3.86$ and $F(3,438)=1.38$, $p>0.01$). In the second experiment, 12 participants listened to speech from which we extracted 346 acoustic edge events that were shown in previous studies to drive the coherence between brain and speech. We analyzed 105 contacts in HG and 59 contacts in STG. We found that these acoustic edges did indeed drive theta-alpha band entrainment that outlasted acoustic stimulation for two cycles in both HG and STG ($F(3,1956)=14.25$ and $F(9,1956)=41.23$, $p<0.01$). Thus, we provide incremental evidence supporting the presence of neural entrainment of oscillations to speech but not to the rhythmic simple sounds in the human HG and STG. Furthermore, these results point out the importance of careful consideration of the task and stimulus design in studies examining the presence of neural oscillations outlasting acoustic stimulation.

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Poster

P433. Auditory Processing: Temporal, Frequency, and Spectral Processing IV

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Program #/Poster #: P433.05

Topic: D.06. Auditory & Vestibular Systems

Support: R01 EB 028155

Title: Nonlinear cortical representation of natural sounds

Authors: *G. R. HAMERSKY, L. A. SHAHEEN, S. V. DAVID;
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Abstract: In everyday hearing, listeners often encounter auditory scenes containing a complex, spectrally overlapping combination of sound sources. Successful perception of a single source, an ability called auditory streaming, requires grouping cues, statistical regularities of natural sounds in the time and frequency domains. Though a substantial number of psychoacoustic studies have described auditory streaming as a perceptual phenomenon, there has been little description of its underlying neural basis, particularly using ethologically-relevant sounds. This study recorded single unit activity in the auditory cortex (AC) of awake marmosets to compare neural responses to natural sounds presented concurrently versus in isolation. Passively listening marmosets were presented with pairs of overlapping natural sound excerpts chosen from ethologically relevant categories of sound textures (backgrounds, BGs) and transients (foregrounds, FGs). Neural responses to overlapping sound pairs (BG+FG) were modeled as linear weighted combinations to the BG and FG sounds in isolation. The model weights showed that responses to the combined sounds were frequently suppressed relative to the individual sound responses. Effects varied across neurons and stimuli, though neural identity, rather than stimulus identity, more strongly predicted the extent of suppression. The linear model also showed that FG responses were more suppressed than BG responses in primary (A1) AC, an effect not observed in non-primary (belt) AC. In addition, responses were often strongly nonlinear and could not be predicted fully from the linear model. Taken together, these results suggest that neural tuning, as well as spectrotemporal cues that distinguish BG and FG categories, contribute to the nonlinearities observed during the presentation of overlapping sounds, an effect that can be explored in more detail by fitting spectrotemporal encoding models and relating spectrotemporal feature selectivity to nonlinear responses to individual natural sounds.

Disclosures: G.R. Hamersky: None. **L.A. Shaheen:** None. **S.V. David:** None.

Poster

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Program #/Poster #: P433.06

Topic: D.06. Auditory & Vestibular Systems

Support: DFG Grant HI 1728/3-1

Title: Auditory corticocollicular neurons display weak and widespread clusters during complex sound processing

Authors: *T. T. X. SCHMITT, S. L. WADLE, K. M. A. ANDREA, J. J. HIRTZ;
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Abstract: The auditory cortex (AC) modulates the activity of upstream pathways in the auditory brainstem via descending (corticofugal) projections. This feedback system plays an important role in the plasticity of the auditory system by shaping response properties of neurons in many subcortical nuclei. Around half of layer 5 (L5) neurons are corticofugal, of which the majority projects to the inferior colliculus (IC). This pathway is involved in processing of complex sounds, auditory-related learning and defense behavior. Partly due to their location in deep cortical layers (500 - 800 µm), population activity patterns of corticofugal neurons within neuronal ensembles of the AC remain poorly understood. We employed two-photon imaging to record the activity of hundreds of L5 neurons simultaneously in anesthetized and awake mice (male and female, postnatal day 45-70). To focus on corticocollicular (CC) neurons, we injected rAAV2-retro (Tervo et al. 2016. A Designer AAV Variant Permits Efficient Retrograde Access to Projection Neurons. Neuron. 92: 372-382) into the mouse IC. Using this approach, we expressed GCaMP7f exclusively in CC neurons and presented animal vocalizations of different species to investigate neuronal ensemble activity. In addition, we mapped the AC using widefield imaging to distinguish between different subfields containing CC neurons. In a further set of experiments, we expressed GCaMP7f in AC, either exclusively in pyramidal neurons or indiscriminately of neuron subtype in awake or anesthetized mice, respectively. By additionally injecting rAAV2-retro-tdTomato into the IC we could image activity of L5 neurons in general, but were also able to identify the subpopulation of CC neurons and analyze their specific network properties.
During complex sound processing, CC neurons intermingle with other L5 neurons and form on average 6 sound-specific clusters. Correlating the activity exclusively of CC neurons revealed that more CC neurons per field of view formed clusters, compared to all L5 neurons. However, CC clusters featured weaker correlations between sound-evoked responses. This finding supports the idea of the “broadcast” function of L5 CC neurons (see for example: Williamson and Polley. 2019. Parallel pathways for sound processing and functional connectivity among layer 5 and 6 auditory corticofugal neurons. eLife. 8:1-21). Furthermore, we found differences in the physical extent of CC clusters between the subfields, implying the existence of subfield-specific tasks for CC neurons during complex sound processing.

Disclosures: T.T.X. Schmitt: None. **S.L. Wadle:** None. **K.M.A. Andrea:** None. **J.J. Hirtz:** None.

Poster

P433. Auditory Processing: Temporal, Frequency, and Spectral Processing IV

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Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P433.07

Topic: D.06. Auditory & Vestibular Systems

Support: NSERC, Grant 201605049

Title: Poisson-wiener prediction of auditory temporal processing in response to artificial and natural sounds

Authors: *C. SABOURIN, X. BAO, S. TAVUKÇU, S. G. LOMBER;
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Abstract: Compromised auditory temporal processing is predictive of impaired speech and language comprehension in patients with auditory neuropathy, cochlear implants, age-related speech deficits, and dyslexia. Temporal modulation transfer functions (tMTFs), descriptions of the sensitivity of auditory neurons to fluctuations in amplitude over time, can be used for the diagnosis and prognosis of auditory neuropathologies. Despite their clinical relevance, the time-consuming and labor-intensive nature of psychophysical measurements of tMTFs, limits their use in routine clinical practice. Recent studies suggest that a system identification method, the Poisson-Wiener theory, can be applied to biologically relevant sounds which often differ based on their temporal patterns, such as speech and other conspecific communication. To assess the predictability of the model in the awake auditory cortex, extracellular recordings of primary auditory cortex neurons were obtained from chronically implanted domestic cats (*Felis catus*, n=2, female) in response to acoustic stimuli: (1) periodic clicks trains to construct tMTFs, (2) Poisson-distributed impulse trains to compute the kernels, and (3) cat vocalizations (purring). For each neuron, Poisson-Wiener kernels were derived and used to estimate prestimulus time histograms, rate modulation transfer functions, and tMTFs specific to that unit. Trends observed in the recordings were consistent with previous findings including observations of monotonic decay and post-activation suppression in first-order kernels. The model was able to accurately capture the tuning properties specific to its unit. Bandpass tuning was the most frequently observed tuning shape for both measured and predicted transfer functions. Presently, greater accuracy is observed for periodic click trains than for purring vocalizations. Our preliminary results suggest the model has the potential to accurately estimate the auditory temporal processing of both natural and artificial sounds. Following successful application to the prediction of conspecific communication, extension of the model to humans has the potential to provide a more efficient and accurate assessment of temporal processing than currently used clinical measures.
This work was supported by NSERC.

Disclosures: C. Sabourin: None. **X. Bao:** None. **S. Tavukçu:** None. **S.G. Lomber:** None.

Poster

P465. Population Dynamics in Visual Cortex

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Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P465.01

Topic: D.07. Vision

Support: NIH-NEI grant: R01EY027402
VRC-NEI grant: P30EY008126

Title: Dioptic stimulation transiently facilitates V1 spiking responses

Authors: *B. MITCHELL[1], K. DOUGHERTY[2], J. A. WESTERBERG[1], B. M. CARLSON[1], L. DAUMAIL[1], A. V. MAIER[1], M. A. COX[3];
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Abstract: Neurons in primate primary visual cortex (V1) play a key role in combining monocular inputs to form a binocular response. Research over the past decades has made great inroads into delineating V1 responses to discrepant (dichoptic) images in the two eyes. Also critical, however, is to decipher how V1 neurons respond to concordance between the two eyes. This question is important since dioptic stimuli occur during many natural viewing conditions. Here, we investigated the extent to which balanced binocular stimulation at (or near) zero disparity modifies V1 contrast responses in awake macaque monkeys. To do this, we presented static gratings to either eye or both at corresponding retinal points while animals passively fixated a central cue. Gratings appeared on screen parafoveally for 250ms and varied in Michelson contrast [0, 0.225, 0.45, 0.9] between trials. We recorded neurophysiological responses to these stimuli using linear multielectrode arrays placed in the parafoveal region of V1. For each V1 unit, contrast response functions were measured to compare between stimulation of one or both eyes. On average, binocular stimuli evoked significantly stronger V1 activity than their monocular counterparts. This binocular facilitation was most prominent during the transient peak (50-100ms) of V1 spiking responses. Notably, higher contrast stimuli attenuated binocular facilitation. This contrast-dependency suggests that response amplification under binocular viewing predominately occurs for low to medium contrast stimuli, reminiscent of the perceptual contrast gain that characterizes binocular vision. Taken together, our results reveal contrast-dependent, transient facilitation in V1 for dioptic stimulation. We will discuss these results in context of down-stream visual processes involved in binocular integration.

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Poster

P465. Population Dynamics in Visual Cortex

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Program #/Poster #: P465.02

Topic: D.07. Vision

Support: R01EY027402

Title: V1 shows facilitation rather than suppression during onset of binocular rivalry flash suppression.

Authors: *B. M. CARLSON[1], M. A. COX[2,1], K. DOUGHERTY[3,1], B. MITCHELL[1], J. A. WESTERBERG[1], A. MAIER[1];
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Abstract: When the two eyes are presented with different stimuli that cannot be perceptually fused, neurons in primate primary visual cortex (V1) tend to fire less than during regular binocular vision (dichoptic cross orientation suppression, or dichoptic suppression). It has been suggested that this broadly suppressed response to interocular conflict destabilizes neuronal population activity in V1. The resulting destabilization might initiate a sequence of perceptual alternations between each eye's view in response to the interocular conflict (binocular rivalry). Here we test this hypothesis by examining neural activity during binocular rivalry flash suppression (BRFS). Binocular rivalry and BRFS are similar paradigms, except that the latter incorporates a period of monocular adaptation before the onset of interocular conflict. Our rationale was that if interocular suppression is required for igniting perceptual alternations more generally, it should be of similar magnitude at the onset of both binocular rivalry and BRFS. To test this prediction, we recorded laminar V1 spiking responses in two awake behaving macaques who passively observed BRFS. As expected, we observed interocular suppression in the spiking responses for simultaneously presented incongruent stimuli. To our surprise, we found facilitation rather than suppression of spiking responses at the onset of BRFS. This finding suggests that dichoptic suppression in V1 does not serve as a general prerequisite for perceptual alternations.

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Poster

P465. Population Dynamics in Visual Cortex

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Topic: D.07. Vision

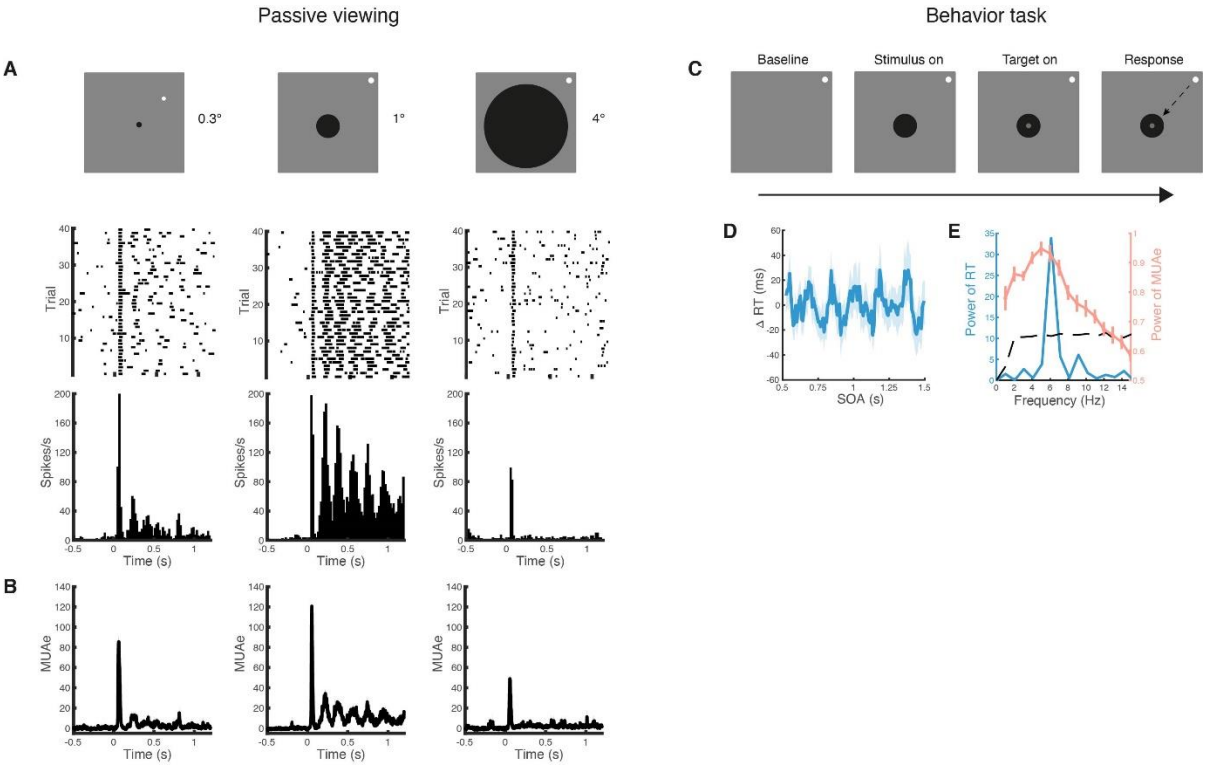
Support: ERC grant Optovision 637638

Title: Stimulus dependence of theta rhythmic activity in primate V1 and its potential relevance for visual perception

Authors: *P. FANYIWI[1], B. AGAYBY[1], R. KIENITZ[2,3], M. HAAG[4], M. SCHMID[1,4];
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Abstract: A growing body of psychophysical research reports theta (3-8 Hz) rhythmic fluctuations in behavioural performance that are often attributed to an attentional sampling mechanism in the brain. Recent studies showed correlates of this sampling in higher-level brain areas[1]. However, it remains unclear whether theta oscillations can be found already in early sensory areas, such as the primary visual cortex (V1). Earlier research suggests that many V1 neurons are tuned to visual stimulus temporal frequencies in the theta range[2]. V1 neurons could therefore act as a filter to generate theta rhythmic spiking activity. To address this question, we recorded multi-unit activity (MUA) from two macaque monkeys passively viewing a static visual stimulus with variable sizes and contrasts. We found that among the visually responsive electrode sites, more than 50 % showed a spectral peak at theta frequencies. Theta power varied with size and contrast of the stimulus. Within each of these stimulus property domains (e.g., size), there was usually a single stimulus value that induced the strongest theta. In addition to these variations in theta power, the peak frequency of theta oscillations increased with increasing stimulus size. Control analysis confirmed that this neural theta rhythm was indeed stimulus induced and did not arise from small fixational eye movements (microsaccades). When the monkeys performed a detection task of a target embedded in a theta-generating visual stimulus, reaction times (RT) also tended to fluctuate at the same theta frequency as the one observed in the neural activity. The present study shows that a highly stimulus dependent neuronal theta oscillation can be elicited in V1 that appears to influence the temporal dynamics of visual perception.

1.Fiebelkorn, I. C. & Kastner, S. A Rhythmic Theory of Attention. *Trends Cogn. Sci.* **23**, 87-101 (2019).
2.Foster, K. H., Gaska, J. P., Nagler, M. & Pollen, D. A. Spatial and temporal frequency selectivity of neurones in visual cortical areas V1 and V2 of the macaque monkey. *J. Physiol.* **365**, 331-363 (1985).



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Poster

P465. Population Dynamics in Visual Cortex

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P465.04

Topic: D.07. Vision

Support: NIH Grant EY023371

Title: Unexpected behavioral synergy from a multidimensionally tuned sensory population

Authors: B. LIU, J. BECK, **L. C. OSBORNE**;
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Abstract: Sensory signals are multiplexed throughout the brain such that spike trains encode information about more than one stimulus feature: sound frequency and intensity, color and orientation, odor identity and concentration, etc. Sensory multiplexing might arise naturally from correlations in the environment, yet even nominally independent stimulus features are jointly represented by sensory neurons. Do multidimensional representations arise solely from anatomic

or metabolic necessity, or is there a computational advantage? Multidimensional tuning functions create ambiguity at the unit level, but also enhance information coding - what we term “stimulus synergy”. When uncorrelated, information about a vector of stimulus features must equal or exceed the summed information about individual features, even when tuning functions for each feature are separable. But the scale of the information boost and its implications for population coding and behavior have not been understood. We analyzed synergy in a sensory population and in a closely coupled motor behavior to understand how multidimensional tuning impacts downstream decoders.
Cortical area MT units are tuned for multiple motion features, e.g. motion direction and speed. We found that MT units encode 30-40% more information about the motion vector than the summed information about direction and speed, a large level of stimulus synergy. Does the brain recover that extra information and, if so, what does that reveal about the nature of visual population decoding? MT provides the visual drive for smooth pursuit, an oculomotor behavior tightly coupled to visual motion. For the eye to track a target, downstream circuits must estimate both its speed and direction. For stimulus synergy to benefit pursuit, MT population response must be synergistic and the brain’s decoders must recover the additional information. Standard population models with homogenous tuning functions do not predict synergistic behavior, yet we observed 20% synergy in smooth pursuit. We investigated populations that produce synergy, and the decoding model that matched both the precision and synergy observed in pursuit. We simulated heterogeneous populations of correlated MT units, varying population size, level of differential correlations, and tuning function diversity. We found that even small deviations from pure homogeneity result in synergistic population coding. To match the level of precision and synergy observed in pursuit, a decoder needs to estimate direction and speed jointly weights derived from the Fisher information matrix and full knowledge of the stimulus dependence of the population noise covariance matrix.

Disclosures: B. Liu: None. **J. Beck:** None. **L.C. Osborne:** None.

Poster

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Topic: D.07. Vision

Support: NSERC Grant No. 210977

Title: Linear alignment of neural modes compensates for drift across trials in early visual cortex population activity

Authors: *C. GODIN[1], J.-P. THIVIÈRGE[2];
[1]Univ. de Moncton, Moncton, NB, Canada; [2]Univ. of Ottawa, Ottawa, ON, Canada

Abstract: In recent years, advances in neural recordings have provided the ability to record from thousands of neurons simultaneously across many areas of the cortex. It is well established that neural activity across large populations of cortical neurons is characterized by low dimensionality. In other words, much of the variance in neural activity can be captured with only a few dimensions, termed *neural modes*, that are extracted by dimensionality reduction techniques. Whereas neural modes have been extensively studied in the motor cortex, less is known about their characteristics in primary visual areas. Here, we extracted neural modes from areas V1 and V2 of three macaque monkeys using a recently developed technique termed frequency-dependent principal components analysis. This technique yields a decomposition of activity into modes that are each delimited to a given frequency range. Animals were shown sets of 8 different orientation gratings across 400 trials per stimulus in the span of 5 different sessions. A Fisher linear decoder was applied to read out stimulus orientation from neural modes of population activity. This decoder attained high accuracy with less than 10 dimensions of neural modes, suggesting that a linear readout can rely on low-dimensional information to extract sensory information. Furthermore, population activity from V2 required fewer dimensions than V1. However, across both V1 and V2 activity, we found a rapid drift in representation among consecutive trials: a decoder trained on a given trial generalized poorly to subsequent ones. This drift could be compensated by a linear alignment of neural modes across trials, effectively "registering" neural modes on a given trial to a reference trial that served as baseline. These findings provide a solution to the stability of a population code for visual processing that rectifies rapid trial-to-trial fluctuations by a fixed linear projection of low-dimensional activity.

Disclosures: C. Godin: None. **J. Thivièrge:** None.

Poster

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Program #/Poster #: P465.06

Topic: D.07. Vision

Support: Israel Science Foundation (grant 2113/19)
European Research Council (ERC-CoG 101001226)

Title: Representational drift in the mouse visual cortex

Authors: D. DEITCH, A. RUBIN, *Y. ZIV;
Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Recent studies have shown that neuronal representations gradually change over time despite no changes in the stimulus, environment or behavior. However, such representational drift has been assumed to be a property of high-level brain structures, whereas earlier circuits,

such as sensory cortices, have been assumed to stably encode information over time. Here, we analyzed large-scale optical and electrophysiological recordings from six visual cortical areas in behaving mice that were repeatedly presented with the same natural movies. Contrary to the prevailing notion, we found representational drift over timescales spanning minutes to days across multiple visual areas, cortical layers, and cell types. This drift had different properties from those of habituation or adaptation. Notably, neural-code stability did not reflect the hierarchy of information flow across areas. Although individual neurons showed time-dependent changes in their coding properties, the structure of the relationships between population activity patterns remained stable and stereotypic. Such population-level organization may underlie stable visual perception despite continuous changes in neuronal responses.

Disclosures: D. Deitch: None. **A. Rubin:** None. **Y. Ziv:** None.

Poster

P465. Population Dynamics in Visual Cortex

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Brain & Behavior Research Foundation #28174
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NIH-NEIR01 EY02881

Title: Linking microscale neural computations to global brain dynamics using deep learning

Authors: *B. SAHOO, A. C. SNYDER;
Brain and Cognitive Sci., Univ. of Rochester, Rochester, NY

Abstract: Brain mechanisms span a wide range of temporal and spatial scales. Large-scale brain activity is captured by EEG with a coarse spatial resolution on the cortical surface and millisecond precision. Whereas, micro-scale neural computations can be accessed using single-neuron recording and local field potentials that have a much finer spatial scale. Even though both these techniques are widespread in cognitive neuroscience research, the inter-relationship between the mechanisms they measure is poorly understood to date. Cognitive processes such as attention, decision making, etc. likely involve an interplay between network-level global coordination among cortical areas and local neural processes. For this reason, an empirical evaluation of the inter-relationship between EEG and spiking activity or LFP would advance our insights into the mechanisms underlying cognition. We recorded spiking activity from area V4 and PFC in the adult monkey brain, simultaneously with scalp-wide EEG activity, while the animal was performing a selective attention task. The change detection task consisted of two

distinct perceptual features i.e. color and motion. The features in the stimulus were tagged with distinct flicker frequencies to evoke distinct neural oscillations encoding individual features. Using novel data analysis tools, we delineated signatures of local neural processing in each area i.e. V4 and PFC, and the inter-areal coordination of information. We used a latent generative model to describe the EEG signals' temporal dynamics and estimated a low-dimensional representation of the high-dimensional EEG signals. Further, we asked how such low-dimensional representations of EEG explain the local vs inter-areal processes. Our results show that within area V4, EEG signals are more aligned with the activity that is targeted to a downstream area i.e. PFC rather than the activity that is primarily local. Additionally, the association between EEG and LFP was enhanced specifically for the feature information that was relevant to the task. Although EEG signals can capture information at a global level due to volume conduction, our work provides a first-in-line insight into the nature of this information. In the future, we will quantify the association between this interplay of EEG and LFP with the animal's behavior.

Disclosures: B. Sahoo: None. **A.C. Snyder:** None.

Poster

P465. Population Dynamics in Visual Cortex

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P465.08

Topic: D.07. Vision

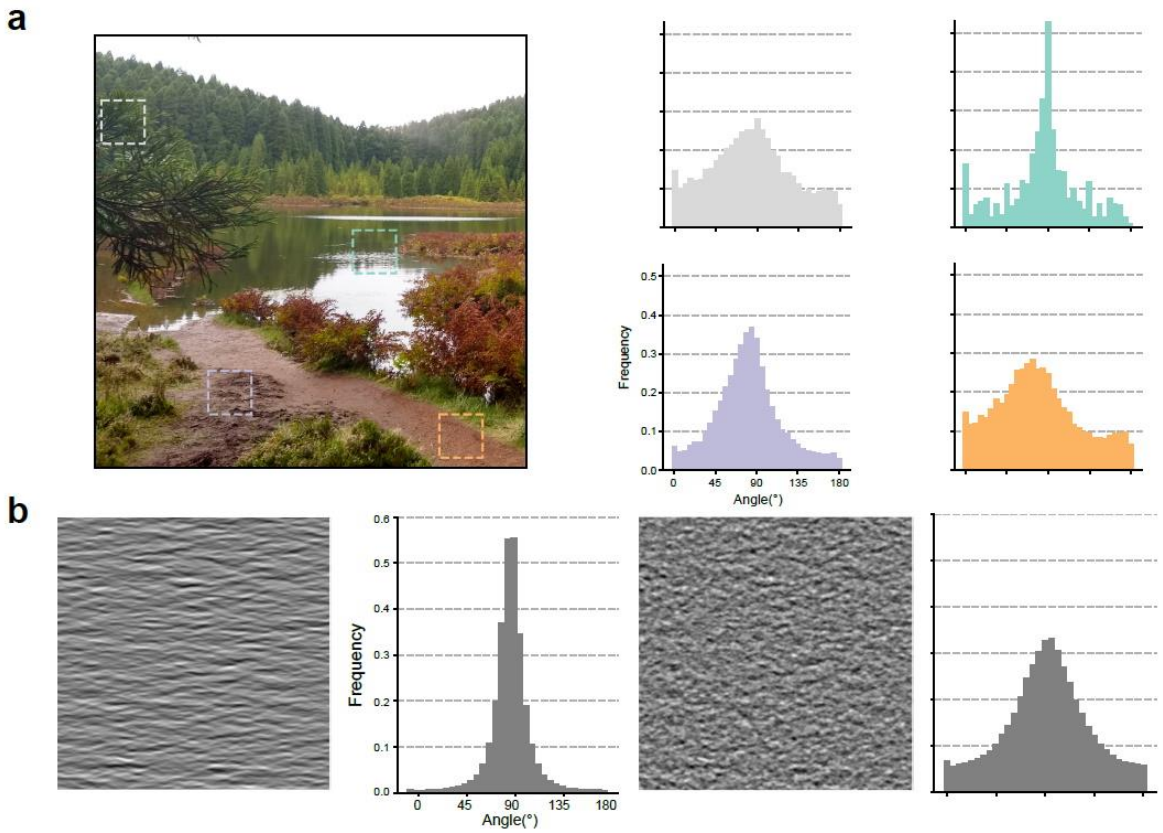
Support: CIHR Grant PJT-148959
ANR Grant ANR-17-CE37-0006
Ecole Doctorale 62 Grant

Title: Modulation of orientation selectivity by orientation precision

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Abstract: The primary visual cortex (V1) processes complex mixtures of orientations to build neural representations of our everyday visual environment. It remains unclear how V1 adapts to the highly volatile distributions of orientations found in natural images. We used naturalistic stimuli and measured the response of V1 neurons to orientation distributions of varying bandwidth. Although broad distributions decreased single neuron tuning, a neurally plausible decoder could robustly retrieve the orientations of stimuli from the population activity at all bandwidths. This decoder demonstrates that V1 population co-encodes orientation and its precision, enhancing population decoding performances compared to sole orientation decoding.

This internal representation is mediated by temporally distinct neural dynamics and supports a precision-weighted description of neuronal message passing in the visual cortex, in line with predictive processing theories.



Supplementary Figure 1. Motion Clouds capture the variety of orientation distributions found in natural images. (**a**) Distribution of orientations found in a natural image (São Miguel Island, Azores, Portugal, picture taken by H.J.L.), retrieved with a Histogram of Oriented Gradients (16x16 cell size, 200^2 pixel subset of the image). (**b**) Two Motion Clouds, oriented at 90° relative to the horizontal axis, with orientation bandwidths of respectively $B_\theta = 15.4°$ and $B_\theta = 36.0°$.

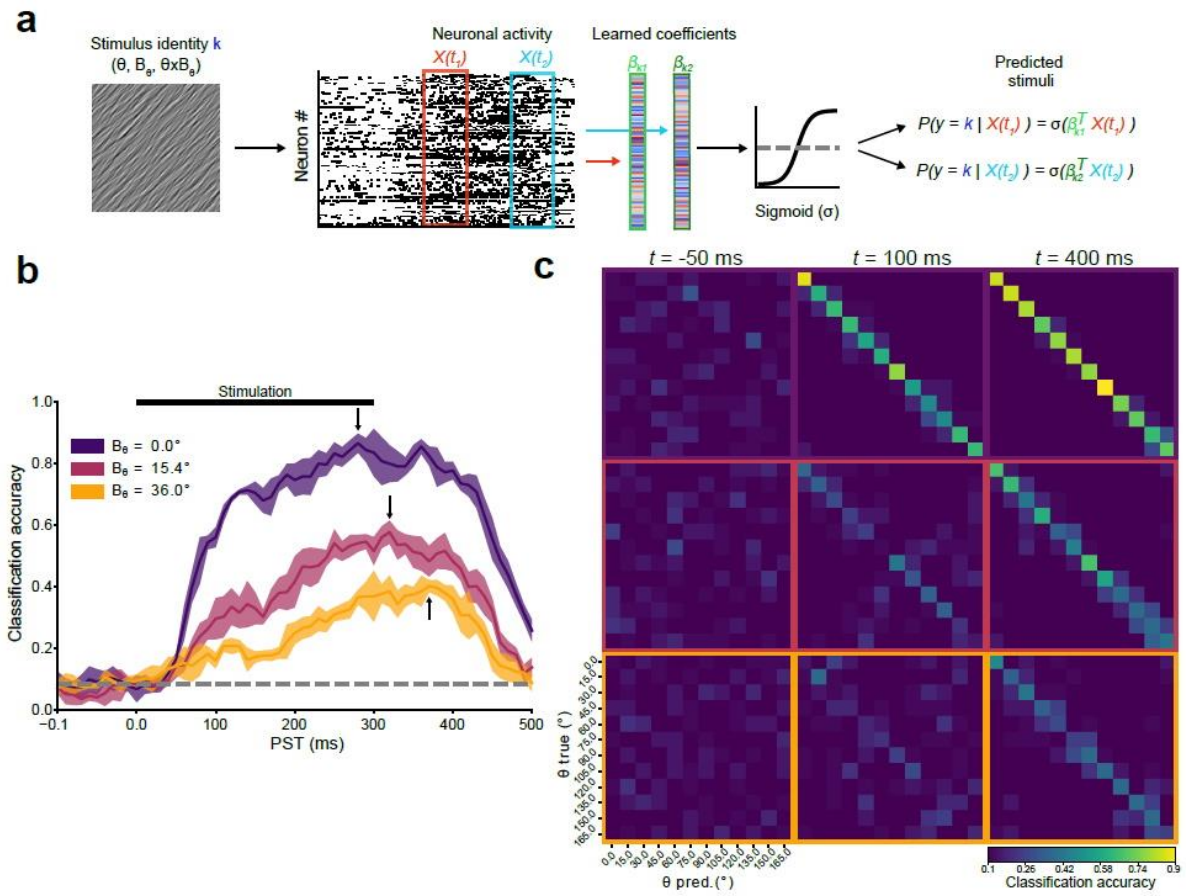


Figure 2. Decoding orientation from population activity. (**a**) Illustration of the decoding model. For each trial, spikes from all single units in a given time window were pooled in a single neural activity vector $X(t)$. A multinomial logistic classifier predicts the identity k of the stimulus in this trial by estimating the probability for different hypothesis y. This is achieved by learning a weight vector β_k which maximizes the accuracy of the prediction $P(y = k|X(t)) \approx \sigma(\beta_k^T X(t))$, where σ is the sigmoid function. The resulting accuracy of this classifier serves as a proxy for the discriminability of the population's neural activity within feature space. (**b**) Time course around the peri-stimulus time (PST) of the accuracy of three independent decoders trained to predict the orientation θ of Motion Clouds for a given orientation bandwidth B_θ. Solid dark line represent the mean accuracy of a 5-fold cross validation and filled contour the SD. Decoding at chance level (here, $\frac{1}{12}$) is represented by a gray dashed line. Arrows indicate the time at which maximum average accuracy was reached. (**c**) Confusion matrices of the decoders at three time points around stimulation time. Values inside the diagonals of each matrix represent the instances of correct decoding, that is, where $\theta(t)_{\text{pred}} = \theta_{\text{true}}$. Each row of matrices is framed with the color code of the corresponding decoder in (b).

Disclosures: H.J. Ladret: None. **N. Cortes:** None. **L. Ikan:** None. **F.Y. Chavane:** None. **C.F. Casanova:** None. **L. Perrinet:** None.

Poster

P465. Population Dynamics in Visual Cortex

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Topic: D.07. Vision

Support: NIH R01EY020950

Title: Coarse-to-fine processing drives the efficient coding of natural images in mouse primary visual cortex

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Abstract: The sequential analysis of information in a coarse-to-fine (CtF) manner is a fundamental processing strategy of the visual system. Previous studies have shown that neurons in the primary visual cortex (V1) of anesthetized animals can process spatial information in a CtF fashion, shifting their spatial frequency (SF) preference from low (coarse) to high (fine) during their response to simple grating stimuli. However, many central questions regarding CtF processing, such as whether it occurs in awake behaving mice and potential computational advantages it may provide animals, remain unexplored. Here, we performed large-scale single unit recordings to characterize CtF processing in both anesthetized and awake mice, determine its developmental profile, and study its role in encoding ethologically relevant natural images. Using high-density multielectrode silicon probes and subspace mapping of receptive fields, we found that the vast majority of V1 neurons from awake adult mice (P40-80) displayed two temporally discrete peaks in their spatiotemporal receptive field, each with distinct SF preferences (which we refer to as “2 peak cells”, 75.3%, n = 195/259, 15 mice). The SF shift between these 2 peaks was large (mean: 2.19 octaves) and nearly always from low to high. Interestingly, anesthesia attenuated the percentage of 2 peak cells (51.2%, n = 99/191, 8 mice) and the average magnitude of their SF shift (0.9 octaves). Additionally, we discovered CtF processing develops postnatally and requires visual experience. The percentage of 2 peak cells was reduced in P17 (57.9%, n = 44/76, 10 mice) and dark reared mice (DR; 58.7%, n = 145/247, 12 mice). The remaining 2 peak cells in P17 and DR mice showed substantially lower average SF shift than that of adult control mice (1.43 octaves for both). Next, we revealed that CtF processing acts to dynamically adjust the SF tuning of V1 as a whole from low-pass to one that represents SFs more uniformly. Finally, we studied how this temporally dynamic cortical SF tuning might affect the processing of natural images with complex SF statistics. Excitingly, we demonstrated that CtF processing reduces redundancy in the neural representation of natural images by shifting the population response away from the high-power, low-SF components of these stimuli. This redundancy reduction led to an increase in the representational efficiency of natural images that did not occur in anesthetized mice with attenuated CtF processing. Collectively, these findings establish a novel, state-dependent, computation of cortical circuitry that develops after vision onset to allow the animal to efficiently encode the spatial statistics of natural images.

Disclosures: R. Skyberg: None. **S. Tanabe:** None. **H. Chen:** None. **J. Cang:** None.

Poster

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Program #/Poster #: P465.10

Topic: D.07. Vision

Support: NIH BRAIN grant: 5U01NS094330

Title: The operating regime of sensory cortex

Authors: *J. J. PATTADKAL[1], B. V. ZEMELMAN[1], I. R. FIETE[2], N. J. PRIEBE[1]; [1]Dept. of Neurosci., The Univ. of Texas at Austin, Austin, TX; [2]Dept. of Brain and Cognitive Sci. and McGovern Inst., MIT, Cambridge, MA

Abstract: Cortical activity results from a combination of external input and internal circuitry. Using calcium imaging in awake marmosets we assay the contributions of the input and internal circuitry in generating cortical dynamics in sensory area MT, known for motion processing. We first uncovered the functional organization in area MT: nearby cells shared their direction preference and the preference varied smoothly along the cortical surface, repeating every 400 microns. We compared the population activity patterns in area MT to three circuit models which show amplification of motion signals, but which differ in the stability of the selective network states. The first model, the homogeneous model (Goldberg et al, 2004), is largely feed-forward input driven and when the external input is unstructured, the network displays only baseline non-selective activity. The second model, the continuous attractor model (Goldberg et al, 2004), uses recurrence to keep generating direction selective output, even in the absence of structured external input. The third model, the transient amplification model (Murphy and Miller, 2009), also uses recurrent excitation to generate strong amplification, but this is rapidly quenched by the dominant inhibition in the network. If internal circuitry is biased towards enhancing direction-specific activity, then we would expect activity patterns in the absence of visual stimuli to resemble those evoked by direction stimuli. We observed such direction selective spontaneous activity patterns in area MT and their occurrence is directly linked to increases in overall network activity. These results are not consistent with the homogenous model, but could emerge from an attractor or transient amplification model. To distinguish which of those frameworks are present in area MT we measured the network dynamics and found that they are rapid, consistent with the transient amplification model. Finally, we performed two additional tests of the attractor model (shifting stimulus direction and coherence) which, like the dynamics measurements, demonstrate that the operating regime is consistent with a transient amplification model. Using both spontaneous and stimulus-evoked conditions, we demonstrate that the internal circuits in sensory areas are geared towards isolating and enhancing feature selective activity using fast non-normal amplification by E and I cells, consistent with the transient amplification model. This network mode allows the circuits to remain sensitive to changes in external environment while amplifying weak signals with internal circuitry.

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Poster

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Topic: D.07. Vision

Support: NIH Grant EY028657
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Title: Origin and functional consequences of noise correlations in mouse V1

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Abstract: Sensory neurons show considerable variability in their response to repeated presentations of a stimulus. In primary visual cortex (V1), this "noise" is correlated between nearby cells, potentially limiting the stimulus information present in the V1 population code. To date, it has been challenging to delineate the sources of these noise correlations in V1 due to the combination of feedforward and recurrent inputs that drive V1 neurons. Recent work in rodent retina has shown that the spatial extent of pairwise noise correlations in retinal ganglion cells differs between scotopic and photopic levels of light adaptation (Ruda et al., Nat. Comm. 2020), though the implications of this finding for downstream processing in V1 remain unclear. By integrating experiment and modeling, we seek to understand the origin and functional consequences of V1 noise correlations by varying the spatial distribution of noise correlations in the retinal input. We employ two-photon fluorescent calcium imaging to measure noise correlations in mouse V1 during repeated presentations of sine wave gratings under scotopic and photopic conditions. Our preliminary analysis shows that the pattern of noise correlations over cortical space in V1 is very similar between the two light levels and can be well described by an exponential decay function over distance. For both light levels, pairwise Pearson correlation values range from 0.15-0.25 for neighboring cells less than 100μm apart, decaying to near zero for cells separated by 500μm or more. To understand these experimental observations, we have constructed a two-layer neural network model in which noise correlations between V1 units are inherited from correlated noise in the inputs from the retina. By varying the spatial extent of noise correlations in the input layer, we find that a substantial component of the spatial pattern of noise correlations in V1 under scotopic and photopic regimes can be explained by a purely feedforward model. However, the spatial extent of non-zero noise correlations in our data is larger than would be possible in the purely feedforward framework. We are adding physiologically constrained recurrent connections between V1 units in the model to explore whether this improves the correspondence between the model prediction and our data from mouse V1. We are also employing this network model to understand how the spatial distribution of noise correlations may limit the fidelity of visual coding of large neuronal populations in V1.

Disclosures: R. O'Shea: None. **X. Wei:** None. **I.M. Nauhaus:** None.

Poster

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Topic: D.07. Vision

Support: NIH Grant EY028626
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Charles H Revson Senior Fellowship in Biomedical Science

Title: Adaptation restructures V1 and V4 representations of crowded displays

Authors: *C. A. HENRY[1], A. KOHN[2];
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Abstract: Adaptation adjusts cortical responses to visual stimuli based on what has been shown in the recent past. This adjustment is thought to tailor the cortical representation to the current environment, but it has been difficult to establish a clear functional improvement of adaptation-induced changes in neuronal responses. We wondered whether this difficulty arose because previous work has focused on stimuli presented in isolation. If adaptation adjusts representations to favor some stimuli in the environment over others, its functional benefit might only be evident when multiple stimuli compete for perceptual awareness. We tested this possibility using visual crowding, whereby stimulus discriminability is impaired by the presence of adjacent distractor stimuli.

We recorded neuronal population responses in anesthetized (V1) and awake, fixating (V1 and V4) macaque monkeys. On some trials, we presented small gratings (targets) in isolation, centered over the population aggregate receptive field. Targets varied in orientation by small (4 degree) steps. On other trials—crowded trials—targets were surrounded by 4-8 distractor gratings of similar orientation. We quantified how well target orientation could be discriminated using the measured activity. The presence of distractors reduced the discriminability of target orientation in single neurons and neuronal populations, with moderate effects in V1 and more pronounced deficits in V4. We then asked how adaptation affected the cortical representation of target orientation in crowded displays. Adapters were high contrast distractor stimuli, either matched or orthogonal in orientation to the distractors of the subsequent test stimuli. Adaptation with matched distractors improved target orientation discriminability in both V1 and V4. Adaptation with mismatched distractors, in contrast, reduced the discriminability of crowded targets. Altered performance arose from an adaptation-induced change in how distractors modulate neuronal responsivity and tuning.

Our results show that adaptation alters neuronal population information for the features of a

target stimulus when it is embedded in an environment with competing stimuli. Thus, the perceptual benefit of adaptation may be most evident in visual environments with multiple, competing stimuli.

Disclosures: C.A. Henry: None. **A. Kohn:** None.

Poster

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Topic: D.07. Vision

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R01-EY028723
Swartz Foundation
Fiona and Sanjay Jha Chair in Neuroscience (J.R.)

Title: Coordination of sparse neural traveling waves (nTWs) in large-scale, multi-region networks of spiking neurons through feedforward and feedback connectivity

Authors: Z. W. DAVIS[1], R. C. BUDZINSKI[2], G. BENIGNO[2], C. C. STEWARD[2], J. H. REYNOLDS[1], ***L. MULLER**[2];
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Abstract: The cortex is never silent: ongoing, spontaneous activity occurs continuously, providing cortical neurons with constantly fluctuating synaptic input (Arieli et al., *Science* 273, 1996). In recent work, we found spontaneous fluctuations in awake cortex are organized into neural traveling waves (nTWs) propagating across single cortical areas (Davis*, Muller*, et al., *Nature* 587, 2020). Spontaneous nTWs modulate excitability as they propagate across the retinotopic map, affecting perceptual sensitivity in a visual detection task. Based on the speed of nTW propagation, we hypothesized the underlying mechanism for these waves is the axonal time delays from unmyelinated horizontal fibers in cortex (0.1-0.6 m/s; Girard et al., *J Neurophys* 85, 2001), which range up to tens of milliseconds on the scale of a 4x4 mm Utah array. We have recently found that the addition of these axonal time delays in a spiking network model shapes asynchronous-irregular (AI) activity into sparse nTWs, where only a small fraction of the local population spikes as a wave passes (Davis*, Benigno*, Fletterman*, et al., *in review*). We call this unique operating mode, where the benefits of the asynchronous-irregular state can coexist with sparse nTWs across the cortex, the “sparse wave regime”. To understand the organization of

multiple spontaneous nTWs generated by the dense horizontal connectivity in each visual area, we now extend this spiking model to include multiple regions. We find that diffuse feedforward and feedback projections can transiently coordinate spatiotemporal activity patterns in multiple regions. These results have several important implications for the structure of ongoing activity in the visual system. Specifically, retinotopic coordination of spontaneous nTWs can boost the response to weak inputs across multiple areas in the visual system. Importantly, because we have previously shown spontaneous nTWs occur during the complex, non-narrowband fluctuations of ongoing activity, the coordination observed in our models provides a novel potential mechanism of inter-area information transfer in the absence of narrowband oscillations.

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Poster

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Topic: D.07. Vision

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MRC Grant MR/K014382/1
Wellcome Trust Strategic Award 101092/Z/13/Z
NIH EY028723

Title: Spontaneous traveling waves in V1 and V4 are retinotopically coordinated

Authors: *Z. W. DAVIS[1], M. H. MOFRAD[2], A. JASPER[3], J. SMITH[4,5], A. KOHN[3], A. J. PARKER[4,6], L. MULLER[2], J. REYNOLDS[1];
[1]Salk Inst., La Jolla, CA; [2]Western Univ., London, ON, Canada; [3]Albert Einstein Coll Med., Bronx, NY; [4]Physiology, Anat. and Genet., Univ. of Oxford, Oxford, United Kingdom; [5]Ernst Strüngmann Inst., Frankfurt am Main, Germany; [6]Inst. of Biol., Otto-von-Guericke Univ., Magdeburg, Germany

Abstract: Understanding the spatio-temporal structure of cortical activity is a fundamental question in neuroscience. Some fluctuations of cortical activity travel as waves across the cortical surface. These neural traveling waves (nTWs) are predictive of local fluctuations in excitability, which have recently been shown to modulate the magnitude of sensory evoked responses to visual motion in area MT of common marmosets (Callithrix jacchus) and impact the perceptual sensitivity of these monkeys as they perform a detection task. It is unknown whether spontaneous nTWs occur independently across cortical areas or are coordinated in their occurrence. We examined simultaneous recordings of chronically implanted multielectrode

arrays in areas V1 and V4 of behaving rhesus and cynomolgus macaques. Spontaneous activity in both V1 and V4 contains nTWs, which are more likely to co-occur than expected by chance. This may imply that nTWs are coordinated to align local excitability topographically across these cortical areas. Owing to differences in the visual representations in V1 and V4, such retinotopic alignment would require an appropriate scaling of wave properties to keep waves in retinotopic register. We found the properties of nTWs in V1 and V4 do indeed differ between the cortical areas according to their relative size in a way that would favor translation of waves in retinotopic coordination. Consistent with such a coordination, we found significant phase similarity during spontaneous nTWs between the two cortical areas as a function of the relative proximity of receptive fields. Our results confirm spontaneous nTWs are observable in visual cortex during active perception and appear to be coordinated across cortical areas with regard to retinotopic representations of visual space.

Disclosures: Z.W. Davis: None. **M.H. Mofrad:** None. **A. Jasper:** None. **J. Smith:** None. **A. Kohn:** None. **A.J. Parker:** None. **L. Muller:** None. **J. Reynolds:** None.

Poster

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Topic: D.07. Vision

Support: NEI Grant R01EY011787
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CONACyT fellowship CVU365863

Title: Long-term stability of neuronal ensembles

Authors: *J. PÉREZ-ORTEGA, T. ALEJANDRE-GARCÍA, R. YUSTE;
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Abstract: Neuronal ensembles are causally related to behaviors, perceptions, and memories, but it is still unknown how stable or flexible they are over time. Here, we used two-photon multiplane calcium imaging to track over weeks the activity of the same pyramidal neurons in layer 2/3 of visual cortex from awake mice (Vglut1::TIGRE2.0 GCaMP6s/f, n = 6) and recorded their spontaneous and visually-evoked responses. Fewer than 50 % of the neurons were commonly active between two sessions. These “common neurons” formed ensembles, most of which lasted for weeks. Stable ensembles preserved ~68 % of their neurons for up to 46 days, and these core neurons had stronger functional connectivity. Stable ensembles were also stably tuned to visual stimulation or modulated by locomotion. Our results demonstrate that neuronal

ensembles can last for weeks and could serve as a substrate for a long-lasting representation of behaviors, perceptions, and memories.

Disclosures: J. Pérez-Ortega: None. **T. Alejandre-García:** None. **R. Yuste:** None.

Poster

P519. Motor Circuits: Anatomy and Function

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Topic: E.03. Basal Ganglia

Support: NSF IOS-1845355
NIH R01NS094450
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Title: Cell-type specific connectivity of sensory and motor cortical input to dorsal striatum

Authors: *B. D. SANABRIA, S. BASKAR, C. R. LEE, D. J. MARGOLIS;
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Abstract: The striatum, a key input structure of the basal ganglia, facilitates sensorimotor integration by sorting information about our environment, decisions, and reward due to its extensive innervation by the cortex and thalamus. A large part of the computational complexity of the striatum derives from the functional organization of its microcircuitry. Excitatory projections from the primary sensory (S1) and motor (M1) cortex overlap in the dorsal striatum (DS). Our lab previously revealed that optogenetic stimulation of S1 corticostriatal synapses preferentially excites striatal parvalbumin-expressing fast-spiking interneurons (FSIs) and reduces behavioral responding during a texture-discrimination task in mice (Lee et al. 2019). In contrast, M1 corticostriatal optogenetic stimulation excites striatal spiny projection neurons (SPNs) and FSIs equally, and promotes behavioral responding in the task. How striatal SPNs and FSIs integrate excitatory signals from S1 and M1 remains unclear. We investigated the hypothesis that there are distinct differences in the anatomical connectivity of S1 and M1 projections onto SPNs and FSIs. We injected AAVs expressing Ruby2 or GFP spaghetti monster fluorescent proteins (sm.FPs) into both S1 and M1 to label corticostriatal inputs. This was followed by ex vivo whole cell current clamp recordings of striatal neurons with biocytin to label single neurons. Slices were processed with immunohistochemistry for the two sm.FPs as well as biocytin. Confocal z-stacks were acquired and 3D reconstructed using Imaris software. Corticostriatal projections from S1 and M1 were observed to course through the striatum and terminate in puncta. Puncta making close approximations to filled neurons were quantified and the corresponding distance from the soma calculated. Preliminary results indicate an increased density of inputs originating from M1 than from S1 on striatal MSNs. These results suggest that

one mechanism underlying the stronger functional innervation of MSNs by M1 than S1 is the increased density of synaptic inputs from M1. Our findings will have fundamental implications for how sensorimotor integration is performed in the striatum.

Disclosures: B.D. Sanabria: None. **S. Baskar:** None. **C.R. Lee:** None. **D.J. Margolis:** None.

Poster

P519. Motor Circuits: Anatomy and Function

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Program #/Poster #: P519.02

Topic: E.03. Basal Ganglia

Support: NIH grants NS100050
NIH grants DA042739
NIH grants DA005010
NSF NeuroNex Award 1707408

Title: Striatal phase locking to the gait cycle during walking

Authors: *L. YANG, S. C. MASMANIDIS;
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Abstract: The striatum plays an important role in regulating voluntary movements, including walking. Previous work shows that striatal neurons encode the start and stop of body movement, as well as body speed. However, since whole-body speed is the result of multiple coordinated limb movements, there is still an incomplete picture of how striatal neurons encode single-limb kinematics during walking (i.e., gait). Here, using high resolution, high-speed video tracking (80 fps), we recorded mice freely walking in a large open arena (60 cm x 60 cm). Simultaneous electrophysiological recordings were performed in the dorsal striatum. The large open field enabled mice to walk at a wide range of speeds. Using open-source machine-learning based behavioral tracking techniques, we successfully extracted the trajectory of each limb's position, which allowed us to examine how neural activity is related to body kinematics at the single-limb level. Drawing on the concept of spike-LFP phase locking from the literature, we used circular analysis methods to test whether individual neurons are locked to specific phases of gait. Indeed, the majority of striatal neurons (~60 %) we recorded were significantly phase locked to gait. While the preferred phase varied across the population, the majority of phase-locked cells showed peak firing around the start of the stride phase. Taken together, these findings show that striatal neurons continuously represent the phase of individual limb movements, suggesting a more nuanced coding scheme than whole-body speed. This work also opens new avenues for exploring how brain activity during walking is disrupted in movement disorder models.

Disclosures: L. Yang: None. **S.C. Masmanidis:** None.

Poster

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Topic: E.03. Basal Ganglia

Support: Fondo de Apoyo a la Investigación de la UASLP (Facultad Medicina): C 14 - FAI - 04 - 02.02
Fondo de Apoyo a la Investigación de laUASLP: C15-FAI-04-47.47

Title: Alterations of striatal cholinergic system in autism model in the rat

Authors: *D. IBANEZ-SANDOVAL[1], R. VELÁZQUEZ CONTRERAS[2], N. SADERI[3], G. FLORES[4], P. E. RUEDA-OROZCO[5], O. IBANEZ-SANDOVAL[6];
[1]Univ. Autónoma de San Luis Potosi, San Luis Potosi, Mexico; [2]Univ. Autonoma de San Luis Potosi, San Luis Potosi, Mexico; [3]Facultad De Ciencias, UASLP, San Luis Potosi, Mexico; [4]Univ. Autonoma de Puebla / Inst. de Fisiologia, Puebla, Mexico; [5]Neurobiologia del Desarrollo y Neurofisiologia, Inst. De Neurobiologia, UNAM, Queretaro, Mexico; [6]Univ. Autonoma De SLP, San Luis Potosi, Mexico

Abstract: Recent studies suggest that the basal ganglia and specifically, the striatum nucleus (NSt) play an important role in autistic spectrum disorder (ASD), characterized by deficits in social communication and interaction, repetitive behavior, and cognitive rigidity. One of the components of the NSt that participates in cognitive processes is the cholinergic interneurons (ChIns). These ChIns are the principal source of acetylcholine (ACh) into the NSt and participate in different processes, such as cognitive behavior, choice of actions, and decision-making. In this sense, diverse studies show that the ACh level decreased in animal models of ASD, at least in the prefrontal cortex (PFC) and the NSt. The decrease of ACh in the PFC was due to an increase in the level of the acetylcholinesterase (AChE, enzyme that degrades ACh) and the low level of choline acetyltransferase (ChAT, the enzyme that synthesizes ACh). Whereas in the NSt, the data suggest that the decrease of the ACh levels was due to an increase in the enzyme AChE, but this is not still clear, whether is due to an increase of AChE activity or exist another possibility, for example, that the ChIns may be affected in the ASD model. This imbalance of ACh levels in the NSt could be lead to alterations in the behavior and changes in the activity of the striatal circuit. Because of this, we study the cholinergic system in the NSt of rats exposed to valproic acid (VPA, as an autism model) for that aim we employed behavior tests and immunohistochemical assay for the enzyme ChAT. So far, the present study shows that the striatal cholinergic system is affected in the rats exposed to VPA. The VPA-exposed animals, exhibited a significant reduction in the number of cells immunoreactive to the enzyme ChAT

(ChAT+), compared with control animals. This decreased was the most important at the dorsolateral striatum. Likewise, we did extracellular recordings of these animals and observed changes in the global activity of NSt, since by giving trains of somatosensory stimulation on a forelimb, we found an exacerbated and non-attenuated response like in control animals. These results could explain part of the hyper response to a sensorial stimulus of patients with ASD.

Disclosures: D. Ibanez-sandoval: None. **R. Velázquez Contreras:** None. **N. Saderi:** None. **G. Flores:** None. **P.E. Rueda-Orozco:** None. **O. Ibanez-Sandoval:** None.

Poster

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Topic: E.03. Basal Ganglia

Support: NIH Grant EY000415

Title: Environment-selective choice of gradient-valued objects is represented in the globus pallidus external segment (GPe) and subthalamic nucleus (STN)

Authors: *A. YOSHIDA, O. HIKOSAKA;
Lab. Sensorimotor Res., Natl. Eye Inst., Bethesda, MD

Abstract: The basal ganglia choose objects whichever their values are flexible or stable (Kim & Hikosaka, 2013). This is controlled by the direct pathway (choose good objects) and the indirect pathway (reject bad objects) (Amita & Hikosaka,2019). However, in real life, there are many objects which may have multiple gradient values (e.g., 5 (best)> 4>3>2>1 (worst)). Moreover, only some of them may be present in a particular environment. For example, objects with the value of 5 and 3 are in environment A, and objects with value 3 and 1 are in environment B. We used many scenes (similar to “Google Map”) as environments and many fractals for objects (target). We first found that the monkey subjects learned many scenes with many objects very quickly (i.e., chose the better object in each scene: rejected value-3 object in scene A, but chose value-3 object in scene B). Can the basal ganglia still control such scene-based object choice? We found task-related neurons in the dorsal part of the caudal GPe (cdGPe). About half of them were excited (excited-type) by the better object (e.g., value-5 in scene A, value-3 in scene B), but not the worse object (e.g., value-3 in scene A, value-1 in scene B). Notably, the excitation to the scene A which implied value-5 object would appear was greater than to the scene B. These results suggest that cdGPe encode two types of information: 1) which object to choose in an environment and 2) how much value will be acquired. Here, a new question arises. How is the activity of the excited-type cdGPe neurons modulated? This may not be caused by the indirect pathway from the striatum because the striatal input to cdGPe is inhibitory. Instead, we

hypothesized that excitatory inputs from subthalamic nucleus (STN) modulate the activity of excited-type cdGPe neuron. Therefore, we recorded from STN. Similar to cdGPe neurons, most STN neurons were excited by the better object, but not the worse object, and the excitation to the scene A was greater than to the scene B (e.g., value-5 in scene A = value-3 in scene B).These results suggest that the STN-cdGPe pathway may be involved in the choice of the better object in any environment and that there is the possibility this STN-cdGPe pathway works in choosing objects, besides the direct, indirect, and hyperdirect pathways.

Disclosures: A. Yoshida: None. **O. Hikosaka:** None.

Poster

P519. Motor Circuits: Anatomy and Function

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P519.05

Topic: E.03. Basal Ganglia

Support: NIH R01MH115030
Tourette Association of America

Title: Exploring corticostriatal dynamics associated with motor control deficits in Neurexin1α mutant mice

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Abstract: Motor control dysfunction is associated with numerous neuropsychiatric and neurodevelopmental disorders, including autism spectrum disorder, schizophrenia, attention-deficit/hyperactivity disorder and Tourette syndrome. Copy number variation of genes encoding for synaptic adhesion molecules, such as Neurexin1 (Nrxn1), have been shown to confer a significantly increased risk for these disorders, however, the underlying neural etiopathology is currently unknown. Recent findings have revealed that loss of Nrxn1α function results in decreased synaptic strength of dorsal prefrontal cortical inputs to the indirect pathway of the dorsal striatum, providing a potential neural mechanism for related motor dysfunction. However, it is unclear how these synaptic deficits neural dynamics in intact in vivo systems. We hypothesize that corticostriatal synaptic deficits from loss of Nrxn1α contribute to neural alterations associated with motor deficits. We employed a novel treadmill based operant task that allows for the comprehensive study of various motor control features including initiation, suppression, task-relevant sensory integration, timing, and action invigoration. Using dual-site in vivo electrophysiological recordings, we were able to obtain local field potential and single unit activity from cortical areas concurrently with their downstream dorsal striatal targets.

Preliminary results suggest genotypic differences in behavioral performance that may correlate with neural changes in corticostriatal circuitry. These findings will provide valuable insight into the neural mechanisms associated with Nrxn1a related motor deficiencies, as well as further elucidating corticostriatal dynamics involved in motor regulation.

Disclosures: S.M. Ferrigno: None. **E. Diaz-Hernandez:** None. **M.F. Davatolhagh:** None. **M.V. Fuccillo:** None.

Poster

P519. Motor Circuits: Anatomy and Function

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Program #/Poster #: P519.06

Topic: E.03. Basal Ganglia

Support: NIH R01NS094450 (DJM)
NSF IOS-1845355 (DJM)
NIH F31NS117093-01A1 (AJY)
NIH NCATS TL1TR003019 (AJY)

Title: Anatomical and functional connectivity of POm-striatal projections

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Abstract: The integration of sensory and motor signals is an essential role of the dorsal component of the striatum, the main input nucleus of the basal ganglia. Despite the widespread excitatory afferents from neocortex and thalamus, the roles of specific cortical and thalamic inputs to this region, such as posteromedial thalamic nucleus (POm), remain poorly elucidated. Thus, it is critical to understand (1) how these POm-striatal inputs influence striatal microcircuitry and (2) where these inputs contact striatal projection neurons (SPNs) and interneurons. Here, we interrogated the anatomical and functional connectivity of thalamic input from POm onto identified striatal cells in the dorsolateral striatum. Channelrhodopsin-2 (pAAV-hSyn-hChR2(H134R)-EYFP) was expressed unilaterally in POm of male and female mice. Ex vivo whole-cell current clamp recordings were carried out on genetically identified dopamine receptor expressing-1 SPNs (D1-SPNs), D2-SPNs, and parvalbumin-expressing interneurons (PV-INs) in the ipsilateral anterior dorsolateral striatum. Identified cells were confirmed via responses to hyperpolarizing and depolarizing current injections including resting membrane potential, half-height width, and other qualitative characteristics (ramp depolarization, spike adaptation frequency, etc.). Our initial results indicate that optogenetic activation (through the 40X objective; ~3.5mW intensity) of POm-striatal terminals readily elicits moderate subthreshold postsynaptic potentials (PSPs) in putative SPNs and suprathreshold responses in

PV-INs. For the single pulse (one 2.5ms pulse, 460nm) protocol at reduced light intensity (~0.3mW; avoid suprathreshold responses), PV-INs exhibited robust subthreshold PSPs (9.12 ± 0.258mV). For the paired pulse (five 2.5ms pulses, 50ms IPI), putative SPNs exhibited moderate PSPs (5.65 ± 0.06mV; ~3.5mW), while PV-INs exhibited robust PSPs (8.89 ± 0.298 mV; ~0.3mW). For the train (25 2.5ms pulses, 97.5ms IPI, 10Hz) protocol, PV-INs also exhibited robust PSPs (8.05 ± 0.413mV; ~0.3mW) with little short-term amplitude changes. Overall, our results appear to show that POm-striatal projections more robustly activate PV-INs (potentially more so than S1/M1 corticostriatal projections) compared to putative SPNs. Also, PV-INs exhibited little short-term amplitude changes to POm-striatal projection activation, which contrasts other striatal-projecting thalamic nuclei.

Disclosures: A.J. Yonk: None. **D.J. Margolis:** None.

Poster

P519. Motor Circuits: Anatomy and Function

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Program #/Poster #: P519.07

Topic: E.03. Basal Ganglia

Support: NIH Grant R01
Einstein Center Neurosciences Berlin

Title: Role of Layer 1 inhibition and apical tuft activity in motor cortex in a decision making task

Authors: *E. MARISTANY[1], J. LEDDEROSE[2], S. SHARMA[2], C. EBNER[1], R. N. SACHDEV[2], D. JAEGER[3], M. E. LARKUM[1];
[1]Humboldt Univ. of Berlin, Berlin, Germany; [2]Charité Universitätsmedizin Berlin, Berlin, Germany; [3]Biol., Emory Univ., Atlanta, GA

Abstract: Anterolateral motor cortex (ALM) is involved in action selection and motor initiation. ALM is linked to basal ganglia, cerebellum and superior colliculus via the motor thalamus input (VM /VA), which has a prominent input to layer 1 (L1) of ALM. The VM input targets interneurons and apical tuft dendrites of pyramidal neurons in L1. Our earlier work showed that activity in VM axons is related to action initiation (Takahashi et al., 2021). Here, we examined whether apical tuft dendrites of pyramidal neurons in were active in a decision-making task, and whether L1 interneurons can influence the behavioral outcome. Mice were trained to attend to air puffs presented to their right or left whiskers, and after a delay, to respond with a lick to the right or left. Additionally, once trained, mice had to relearn a contextual change within a session. More concretely, right puffs were not anymore associated with right licks but as left puffs, with left licks. First, we characterized the animal's behavior by tracking different body parts (nose,

paws, tongue) during the task. We found several movement patterns during the preparatory period, which differed from each other based on trial type and outcome. We imaged Ca^{2+} activity of pyramidal cell apical dendrites in L1 and show that a proportion of dendritic activity is selective for trial type -- right or left lick -- and epoch, i.e., lick delay or response window. Next, we used optogenetics to examine whether activating L1 interneurons biased performance. To our surprise, in the control behaviour paradigm, there was no effect of activating L1 interneurons on the performance of the learned task. However, in the contextual relearning paradigm we found that L1 inactivation played an important role for the mice's capacity to adapt to the new task. To further understand how apical dendrites compute task relevant information, we are currently combining Ca^{2+} imaging with glutamate release imaging (iGluSNFRs) and are directly targeting apical dendrites during the task to test their influence on action selection.

Disclosures: E. Maristany: None. **J. Ledderose:** None. **S. Sharma:** None. **C. Ebner:** None. **R.N. Sachdev:** None. **D. Jaeger:** None. **M.E. Larkum:** None.

Poster

P668. Neural Systems V

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Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P668.01

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant T32 DA007268
NIH Grant R01 DK106188

Title: Input specificity and mechanisms underlying junk-food induced increases in calcium permeable AMPARs, and roles in food motivation

Authors: *T. L. FETTERLY[1], C. R. FERRARIO[1,2];
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Abstract: Alterations in brain reward centers, including the Nucleus Accumbens (NAc), contribute to over-eating and craving that drives the development and persistence of obesity in humans. In rats, cue-triggered food-seeking is enhanced in obesity-prone males and this behavior is mediated by calcium-permeable AMPA receptors (CP-AMPARs) in the NAc. After consuming sugary, fatty junk-food, a rapid and persistent increase in both NAc CP-AMPAR synaptic transmission and surface expression is observed in male obesity-prone, but not obesity-resistant rats. Interestingly, the synaptic incorporation of CP-AMPARs requires a brief 24 hr junk-food deprivation period in which rats are returned to ad lib standard chow. However, it's unclear in which inputs to the NAc this increase in CP-AMPARs is occurring, and what mechanism may be driving insertion of these receptors during the junk-food and subsequent deprivation period. The NAc receives glutamatergic input from a wide variety of brain regions,

including direct connections from the medial prefrontal cortex (mPFC) and basolateral amygdala (BLA). To differentiate these inputs, we used whole-cell patch clamp recordings in combination with a viral optogenetic strategy to selectively record glutamatergic transmission from either the mPFC or BLA inputs to NAc neurons. Following stereotaxic injection of pAAV-CamKII-Chronos-GFP into either input, we prepared ex vivo brain slices containing the NAc and then recorded optically-evoked AMPAR currents in medium spiny neurons. The contribution of CP-AMPARs to AMPAR transmission was determined using naspm (200 µM), a CP-AMPAR selective antagonist. Initial data show that eating junk-food (10 days, 24 hr deprivation) enhanced CP-AMPAR transmission in mPFC-to-NAc inputs but did not alter transmission in BLA-to-NAc inputs. Thus, results suggest that junk-food induced enhancements in CP-AMPARs occur preferentially at mPFC inputs. We hypothesized that the increase in CP-AMPARs following junk-food/deprivation may be due to decreased mPFC activity. In ongoing experiments, we are assessing whether decreasing activity in mPFC inputs is sufficient to cause increases in synaptic CP-AMPARs. Finally, given the role of NAc CP-AMPARs in cue-triggered motivation, we are also determining how junk-food alters cue-potentiated feeding and cue-triggered food-seeking. In the future we hope to understand the specific role of CP-AMPARs in the mPFC-to-NAc inputs in regulating these behaviors.

Disclosures: T.L. Fetterly: None. **C.R. Ferrario:** None.

Poster

P668. Neural Systems V

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Program #/Poster #: P668.02

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIDDK F32 DK126355
NARSAD
McDonnel Center for Cellular and Molecular Neurobiology

Title: Investigating accumbal firing during hedonic food seeking in obesity

Authors: B. A. MATIKAINEN-ANKNEY[1], A. V. KRAVITZ[2];
[1]Washington Univ. in St. Louis, Saint Louis, MO; [2]NIDDK, Natl. Inst. of Hlth., Bethesda, MD

Abstract: Obesity is a leading cause of preventable death in the United States and prevalence of obesity is growing worldwide. Despite widespread knowledge of the health risks associated with obesity, successful treatments remain elusive. One reason may be because we do not understand how obesity changes the brain. Though work over the past several decades has advanced our understanding of how obesity alters brain activity in areas linked to homeostatic feeding, less is known about how motivational components of feeding are altered in obese states. Here, we

investigated how obesity altered neural firing in a brain region linked to motivation and palatable food seeking - the nucleus accumbens. Using chronic in vivo electrophysiological recordings on a novel behavioral platform, we asked how neural activity was affected by obesity as mice seek food and exert effort to acquire food rewards. Obese mice exhibited increased effort output during food seeking relative to lean mice. Accumbal firing was significantly higher during approach and lower during lever press in obese mice relative to lean, suggesting that accumbal encoding of food seeking is dysregulated in obesity. Furthermore, analysis of spontaneous and evoked units suggests that this may be due to aberrant burst firing in the accumbens of obese mice relative to lean. These findings implicate the nucleus accumbens as an in vivo target of persistent synaptic modifications during obesity.

Disclosures: B.A. Matikainen-Ankney: None. **A.V. Kravitz:** None.

Poster

P668. Neural Systems V

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Program #/Poster #: P668.03

Topic: G.02. Reward and Appetitive Learning and Memory

Support: Washington Research Foundation Postdoctoral Fellowship (ERS)
NIDA 4R00DA045662-02 (SAG)
NARSAD Young Investigator Award 27082 (SAG)

Title: Brain-wide cellular ensembles of persistent to incubated relapse to food craving

Authors: *E. R. SZELENYI[1,2], R. MADANGOPAL[3], B. T. HOPE[3], Y. SHAHAM[3], S. A. GOLDEN[1,2];
[1]Biol. Structure, [2]Ctr. of Excellence in Neurobio. of Addiction, Pain, and Emotion (NAPE), Univ. of Washington, Seattle, WA; [3]Behavioral Neurosci. Res. Br., IRP/NIDA/NIH, Baltimore, MD

Abstract: Background: Obesity is a costly and unyielding public health issue faced by modern society. Currently, successful treatments for obesity focus on behavioral modifications where individuals abstain from highly palatable foods, but often result in relapse due primarily to food craving. This is analogous to the well-studied phenomenon of incubation of drug craving, where abstinence duration sensitizes drug relapse vulnerability. Yet, the brain-wide neural mechanisms underlying this process are poorly understood. Here, we establish a novel operant conditioning procedure for the study of palatable food seeking and incubation of craving. We combine this approach with a cellular resolution whole-brain imaging pipeline to survey the brain-wide neural ensembles engaged by persistent and incubated behavioral relapse. **Methods:** Male outbred CD-1 mice were trained to lever press for highly palatable food pellets (68% carbohydrates) using a

fixed-interval 20-second reinforcement schedule over 7 daily, 60-minute sessions. Following 1, 15, or 60 days of abstinence, mice underwent a 60-minute non-reinforced test trial in the training context using a between-subjects design. Whole-brain activation maps of each group of animals, including controls, were generated using iDISCO+ Fos whole-mount staining and light sheet fluorescent microscopy image acquisition. Volumetric registration, regional annotation, and cell segmentation were performed with ClearMap open-source software and custom python scripts. **Results:** Subjects reliably acquired palatable food self-administration and were tested on days 1, 15 and 60 for relapse. Persistent relapse was seen on days 1 and 15, and incubation was observed on day 60. Brain-wide Fos+ activation maps revealed a progression of regions recruited by food craving in an abstinence duration-dependent fashion, that originated and persisted at early time points (e.g. infralimbic cortex, claustrum, endopiriform cortex, basomedial amygdala, dlPAG) and expanded to nearby (e.g. sensory cortices, basolateral amygdala,) and adjacent (e.g. lateral hypothalamus, olfactory tubercle, entorhinal cortex, pontine reticular nucleus) regions at 60-day, concomitant with behavioral incubation. **Conclusion:** Incubation of food craving ensembles are generated through a "conserved recruitment" effect, whereby persistent relapse brain areas sensitize in activation over time - with a recruitment of neighboring structures observed during behavioral incubation. These results provide critical insight into the neural mechanisms driving relapse to palatable food and offer novel therapeutic entry points in the treatment of obesity.

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Poster

P668. Neural Systems V

Location: Virtual Only

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Program #/Poster #: P668.04

Topic: G.02. Reward and Appetitive Learning and Memory

Support: CTRHB at FBRI P/F grant

Title: Macronutrient control of food reward

Authors: A. HARTLE[1], J. SISCO[1], K. RUNYON[1], K. MARSCHALKO[1], A. DIFELICEANTONIO[2], ***W. HOWE**[1];
[1]Virginia Tech. Neurosci. PhD Program, Blacksburg, VA; [2]FBRI at Virginia Tech., Roanoke, VA

Abstract: Neural representations of the sensory/hedonic qualities of a food, as well as caloric density are known to be key determinants of food attractiveness. Recent studies have also shown that when controlling for caloric density and hedonic impact, food items made of combinations of fats and carbohydrates ("combo") are associated with greater monetary value and evoke

unique patterns of activity in striatal reward circuits compared to foods made either macronutrient alone. Given previous data demonstrating that post-ingestive signals of both fat and carbohydrate intake can modify activity in midbrain dopamine (DA) populations, we hypothesized that previous exposure to combo foods amplifies future consumption-evoked striatal DA release, and this enhances the attractiveness of these foods. Here, we examined the impact of single and combo macronutrient foods on reward seeking and DA release in mice. We found that when given free access to familiar food stimuli made of carbohydrate, fat, or combo (equal parts fat and carbohydrate), mice consumed nearly 4 times as much combo compared to either macronutrient alone (n=16). This preference was observed in both sexes and was independent of satiety. Measurements of DA release using DLight and fiber photometry revealed a near doubling of phasic DA release events in the nucleus accumbens during consumption of the combo compared to isocaloric amounts of the fat or carbohydrate stimuli (n=7, $p<0.001$). Together, these results suggest that the macronutrients in a food modify its effect on the DA release, and this contributes to its attractiveness. Future experiments will assess the ability of these different foods to modulate DA in the dorsal striatum, activity in the broader hindbrain-to-midbrain circuitry they recruit, as well as their ability to modulate motivated food seeking.

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Poster

P668. Neural Systems V

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Program #/Poster #: P668.05

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant R01DA042499
NIH Grant R01DA041781
NIH Grant R01DA045463

Title: Investigating the role of the dorsal hippocampus in driving reinforcing behaviors.

Authors: *K. IBRAHIM[1], N. MASSALY[1], H. E. FRYE[1], H.-J. YOON[1], R. SANDOVAL[1], A. ZEC[1], S. PATHIRANAGE[1], O. IDOWU[1], S. WILLIAMS[1], T. KASH[2], A. V. KRAVITZ[1], J. MORON-CONCEPCION[1];
[1]Washington Univ. St. Louis, St. Louis, MO; [2]UNC- Chapel Hill, Chapel Hill, NC

Abstract: The dorsal hippocampus (dHPC) has been repeatedly shown to play an important role in the formation, maintenance, and retrieval of contextual, spatial, and drug-induced memories. Interestingly, pharmacologically inhibition of the nucleus accumbens (NAc) also disrupted contextual and spatial memories, suggesting the presence of functional coordination between the

dHPC and NAc in maintaining memory formation (Xia et al. 2017, Ito et al., 2008). Recently, Trouche (2019) has highlighted the critical role of the dCA1 CaMKII+ projecting neurons to the fast-spiking interneurons in the NAc shell in retrieving reward-induced memories. While silencing this projection may impair memory retrieval, the sufficiency of the dCA1 activation in driving reinforcing behaviors has yet to be determined. To investigate the role of dHPC activation in reinforcement, we used both un-cued real-time place test (RTPT) and self-stimulation (SS) paradigm to determine the active seeking of operant dHPC photo-stimulation in a more translational model. Channelrhodopsin (ChR2) or control virus was expressed in dHPC CaMKII+ neurons of C57/BL6 male mice before implantation of optic fibers. In both experiments, we observed that the mice that expressed ChR2 not only prefer stimulation of dHPC neurons but also actively seeking for stimulation. To investigate the effect of neuronal activity in the NAc with the activation of dHPC excitatory neurons, we expressed GcAMP (or control virus) in the NAc together with ChR2 (or control virus) in the dHPC before the implantations of fibers. We observed that stimulation of dHPC CaMKII+ neurons in the home cage induced an increase in calcium transient, a marker for cellular activity, in the NAc. Pairing this with the SS paradigm, we determined that the increase in neuronal activity of the NAc is due to the activation of dHPC and not the cue. Lastly, we narrowed down our focus in the NAc and investigate the effect of dHPC activation on dynorphin- and enkephalin-containing neurons. Currently, our data shows that the dHPC excitatory neurons are not only necessary for the retrieval of contextual memories but also encode reinforcing values that may trigger reward-seeking via the activation of the Nac shell.

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Poster

P668. Neural Systems V

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Program #/Poster #: P668.06

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIH Grant DA033386
NIH Grant DA042362
Mind Science Foundation BrainStorm Research Award

Title: Delays to reward delivery enhance the preference for an initially less desirable option: role for the basolateral amygdala and retrosplenial cortex

Authors: *M. J. LEFNER[1], A. P. MAGNON[1], J. M. GUTIERREZ[1], M. R. LOPEZ[3], M. J. WANAT[2];

[2]Neurosciences Inst., [1]Univ. of Texas at San Antonio, San Antonio, TX; [3]Biol., Univ. of Texas At San Antonio, San Antonio, TX

Abstract: Reward-based decisions are driven by cost-benefit analyses. A purely economic decision should be influenced solely by the *future* costs associated with earning a reward. However, evidence across species demonstrate that *past* costs (i.e. sunk costs) influence decision-making policies. Sunk costs can result in subjects persisting in the current reward-seeking action despite better options, which suggests that sunk costs increase a reward's value. The effect of sunk costs on behavior has been primarily studied in tasks in which choosing one course of action comes at the expense of an alternative outcome. However, this approach cannot determine if sunk costs increase the value of the chosen outcome and/or decrease the value of the alternative outcome.

To address this, we developed a rodent task to determine how sunk temporal costs prior to a reward delivery subsequently alters reward consumption and preference. We first identified the relative preference between different flavored food rewards in a free-feeding test. Rats then underwent training sessions in which the initial less preferred reward was delivered after long delays (high temporal cost), while the initial preferred reward was delivered after short delays (low temporal cost). The impact of these training sessions on reward value (consumption) and preference was then assessed in a second free-feeding test performed after training. We found that high temporal costs imposed during training sessions subsequently increases the value and relative preference for the initial less desirable reward.

We probed whether this change in reward preference involved neural systems that contribute to timing, reward valuation, and learning. Pharmacological manipulations and site-specific lesions were performed to examine the potential involvement of the dopamine system, the orbitofrontal cortex (OFC), the basolateral amygdala (BLA), and the retrosplenial cortex (RSC). The change in reward preference was unaffected by systemic dopamine receptor antagonism or OFC lesions. In contrast, lesions of the BLA or the RSC prevented the enhanced consumption and preference for the initial less desirable reward. These findings demonstrate that both the BLA and RSC participate in how sunk temporal costs alter reward value and preference. Preliminary photometry recordings were performed to determine when these regions are responsible for updating preference. Taken together, our data highlights a previously unappreciated role for the BLA and RSC in mediating enhanced preference for a less desirable reward that follows a long temporal delay.

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Poster

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Program #/Poster #: P668.07

Topic: G.02. Reward and Appetitive Learning and Memory

Support: DA042934 (NIDA)
DA042934-03S1 (NIA)

Title: Medial prefrontal cortex neural encoding in an Alzheimer's disease rat model during an outcome devaluation task

Authors: *T. J. SLOAND[1,2], M. NIEDRINGHAUS[1], E. A. WEST[1,2];
[1]Cell Biol. and Neurosci., Rowan Univ. SOM, Stratford, NJ; [2]Rowan Univ. GSBS, Stratford, NJ

Abstract: Alzheimer's disease (AD) is characterized by the accumulation of neuropathological markers (Aβ plaques, tau pathology, and neuronal loss) and profound memory loss. However, additional learning and cognitive deficits may also contribute to changes in quality of life in AD patients. AD rats (TgF344-AD) expressing mutant amyloid precursor protein and presenilin-1 exhibit age-dependent (18-24 months) AD pathology (i.e., plaques, tau pathology, oligomeric Aβ and neuronal loss), but show hypofunction within the rodent medial prefrontal cortex (mPFC) at an earlier timepoint (6 months). We have shown that neural activity during learning in the mPFC predicts and is necessary for the ability of rats to suppress behavior following outcome devaluation. Thus, we aimed to determine if AD rats would show aberrant neural activity and/or behavioral responding to reward predictive cues following outcome devaluation. Here, we recorded mPFC (prelimbic and infralimbic) *in vivo* electrophysiological activity during an outcome devaluation task in rats. AD rats (n=3) and wild-type litter mate controls (n=4) were presented with two distinct cues as conditioned stimuli (CS+; predicting a sugar or food pellet) and two cues that did not predict a reward (CS-); 10 trials each. After 10 sessions, rats underwent a devaluation procedure to induce a conditioned taste aversion (LiCl, i.p., 0.3 M; 10 ml/kg) to the sugar pellets. Rats were then tested on the same Pavlovian task (under extinction) to evaluate their ability to avoid CS+ associated with the devalued outcome. Control rats spent less time in the food cup during the CS+ associated with the devalued outcome (12.6%) compared to the CS+ that predicted the non-devalued outcome (18.5%). AD rats did not differ in the time spent in the food cup during the devalued CS+ compared to the non-devalued CS+ (13.0% vs. 12.3%, respectively). Electrophysiological recordings in the mPFC revealed distinct neuronal populations that were excited or inhibited during the cues (classified as "phasic"). Interestingly, phasic neurons in AD rats were exclusively excited to the cues, where the phasic neurons in control animals consisted of both excited and inhibited neurons. There was no difference in % of neurons excited (EXC), inhibited (INH), or nonphasic (NP) to the non-devalued CS+ (27.1% INH, 18.8% EXC, 54.2% NP) compared to the devalued CS+ in control rats (27.1% INH, 12.5% EXC, 60.4% NP; n=48) and no difference between the non-devalued CS+ (0.0% INH, 34.4% EXC, 65.6% NP) or the devalued CS+ (0.0% INH, 34.4% EXC, 65.6% NP; n=32) in AD rats. In the future, we will correlate these neurophysiological characterizations with the behavioral deficits in AD rats.

Disclosures: T.J. Sloand: None. **M. Niedringhaus:** None. **E.A. West:** None.

Poster

P668. Neural Systems V

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Program #/Poster #: P668.08

Topic: G.02. Reward and Appetitive Learning and Memory

Support: UT Dallas Startup
UT System Rising STARs Award

Title: Rats self-administer right, but not left, cervical vagus nerve stimulation

Authors: J. BROUGHER[1], U. AZIZ[1], M. CHATURVEDI[1], A. JULES[1], I. SHAH[1], ***C. THORN**[2];
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Abstract: The vagus nerve is an important carrier of sensory information from the gut and other visceral organs to the central nervous system. Emerging evidence suggests that activation of ascending projections through the right, but not the left, nodose ganglion strongly modulates reward-related behavioral responses and striatal dopamine release (Han et al., 2018). Building on these recent findings, we hypothesized that, if right vagal activation conveys visceral information to the mesolimbic DA system more effectively than left vagal activation, then standard electrical stimulation of the right vagus nerve (r-VNS) may drive self-administration (SA) more readily than VNS that is traditionally delivered on the left side (l-VNS). Here, 14 adult male Long-Evans rats were randomly assigned to r-VNS (n = 7) or l-VNS (n = 7) treatment groups and implanted with cervical VNS electrodes around the targeted nerve prior to undergoing a VNS SA assay. After recovery, rats were habituated to press a lever to receive food reward. After these initial sessions, food was removed and 5 VNS SA sessions were given, in which lever pressing resulted in the immediate presentation of a visual cue paired with delivery of VNS. We found that rats in the r-VNS group quickly learned to self-administer r-VNS, but that l-VNS was not sufficient to sustain lever pressing behavior. After 5 days of SA, rats were given 5 extinction (EXT) sessions, during which VNS and visual cue were no longer delivered. During EXT, r-VNS treated rats stopped engaging in the lever press task. Following EXT, rats were given an initial session of reinstatement (R1) in which the visual cue (but no VNS) was delivered following each lever press. Rats in the r-VNS treatment group exhibited significant cue-induced reinstatement of lever pressing during R1, suggesting strong reinforcement of reward-predictive stimuli via engagement of mesolimbic dopaminergic nuclei. After cue-only reinstatement, rats received an additional reinstatement session (R2) in which VNS was again paired with lever pressing. To ensure that both r-VNS and l-VNS groups received equal amounts of stimulation during R2, each rat in the l-VNS group was yoked to a rat in the r-VNS group and VNS was simultaneously delivered to both rats contingent on the behavior of the r-VNS rat. Ninety minutes after R2, rats were sacrificed and c-Fos expression was subsequently examined in the VTA and SNc. c-Fos expression in both TH+ and TH- cell populations in both regions was significantly elevated

following r-VNS treatment compared to l-VNS. Taken together, our behavioral and histological results suggest that r-VNS activates mesolimbic reward circuits more effectively than l-VNS.

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Poster

P668. Neural Systems V

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P668.09

Topic: G.02. Reward and Appetitive Learning and Memory

Support: R01-DA044315 (L.S.Z.)
T32NS099578 (J.E.E.)
ADAI-1019-17 (B.J.)

Title: Dissociable activity dynamics in projection-specific midbrain dopamine subpopulations contribute to reward association and motivation

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Abstract: The midbrain dopamine (DA) system regulates reinforcement learning and motivation. Anatomical and functional heterogeneity among ventral tegmental area (VTA) DA neurons with defined projection patterns to the nucleus accumbens (NAc) are proposed to distinctly contribute to these processes. Cell-type specific targeting of genetically isolated VTA DA populations with optogenetic manipulations during classical and instrumental conditioning showed that DA neurons with distinct projections to NAc core and shell subregions differentially regulate reward association and reward motivation. While these studies established the necessity and sufficiency of NAc core and shell-projecting DA subpopulations for learning and motivation, how the activity of these neurons might contribute to the distinct phases of acquisition, extinction, and reinstatement of an appetitive behavior remains unresolved. Here, we selectively expressed GCaMP6m in genetically isolated VTA DA populations with divergent projection patterns to subregions of the NAc and utilized fiber photometry to record intracellular calcium dynamics across these different phases of behavior. We observed that both populations are activated by cues, actions, and rewards during instrumental behavior. However, shell-projecting VTA DA neurons display prominent ramping of calcium signals leading up to the lever press (action) that develops rapidly during the acquisition of the action- and cue-reward association. These ramps are absent in NAc core-projecting VTA DA neurons. Further, the NAc shell-projecting DA population maintains an increase in the calcium signal between the action and presentation of the reward-predictive cue, and between the cue and the reward delivery. In

contrast, these signals in the core-projecting population return to baseline between the discrete events. During unexpected reward omission, the NAc core-projecting population displays temporally discrete decreases in calcium signals that is not observed in the NAc shell-projecting cells. Our data demonstrate that calcium signals at the level of the cell bodies of NAc core- and NAc shell-projecting VTA DA neurons are distinct and suggest that NAc core-projecting dopamine neurons provide temporally discrete information about actions, cues, and outcomes to facilitate reward association while NAc shell-projecting VTA neurons provide sustained signals through these events to promote motivated behavioral responses. These results provide further evidence that the midbrain DA system contains functionally distinct subpopulations for the regulation of learning and motivation.

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Poster

P726. Addictive Drugs: Human Neural Mechanisms

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Program #/Poster #: P726.01

Topic: G.09. Drugs of Abuse and Addiction

Support: NCCIH Grant R01AT010627

Title: Cue-reactivity, reappraisal and savoring in human heroin addiction- an fMRI study

Authors: *Y. HUANG[1], A. O. CECELI[1], S. KING[1], G. KRONBERG[1], P. MALAKER[1], E. GARLAND[2], N. ALIA-KLEIN[1], R. Z. GOLDSTEIN[1];
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Abstract: Drug addiction is characterized by impaired Response Inhibition and Salience Attribution (iRISA), including enhanced neurophysiological/corticostriatal reactivity to drug vs. nondrug cues and reinforcers. Cognitive strategies to reinterpret drug cues and competing nondrug reinforcers (reappraisal of drug, savoring of food) have been employed to modulate emotional reactivity and normalize incentive salience. However, drug cue reactivity has been less extensively studied in opioid addiction, and whether reappraisal and savoring may normalize cue reactivity-related brain function remains unresolved. To this end, 32 participants (age = 40.24 ± 8.82; 25M) with heroin use disorder (HUD) underwent a novel cue reactivity fMRI task with instructions to passively view heroin, food, and neutral cues, reappraise heroin cues and savor food cues. Results were analyzed by FSL and cluster-corrected to $p<.05$. We hypothesized that heroin vs. food/neutral cues would upregulate corticostratal reward networks, while reappraisal and savor conditions would enhance prefrontal function related to cognitive control [e.g., dorsolateral prefrontal cortex (dlPFC) and inferior frontal gyrus (IFG)]. Although subjective

ratings revealed significantly higher craving of food vs. heroin [$F(1,31)=23.09$, $p<.01$], passive drug vs. neutral/food cue viewing was associated with increased activations in the reward network [midbrain, striatum and ventromedial PFC (vmPFC)] and cognitive control network (bilateral IFG/dlPFC), among other regions [e.g., posterior cingulate cortex (PCC), amygdala, hippocampus] (drug>neutral at $Z>3.1$; drug>food at $Z>2.3$). Compared to their respective passive viewing conditions, both reappraising heroin and savoring food cues showed increased activation in the left IFG, along with left dlPFC activation during savoring ($Z>3.1$), and higher activation in the bilateral IFG and PCC during reappraisal compared to savoring ($Z>3.1$). Additionally, the vmPFC showed higher activations during passive drug cue viewing than reappraising ($Z>2.3$). Our novel task support the iRISA model, revealing pronounced corticostriatal activity to drug vs. nondrug cues in HUD. Additionally, increased IFG activity during drug reappraisal and both IFG and dlPFC during food savoring alludes to the suppression of drug cue reactivity via top-down cognitive control processes; the vmPFC may facilitate this process by decreasing the value assigned to the drug cues during reappraisal. The neural mechanisms of drug cue reactivity and its suppression via reappraisal and savoring in HUD may highlight target brain areas for cognitive and neuromodulatory interventions.

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Poster

P726. Addictive Drugs: Human Neural Mechanisms

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Support: Commonwealth of Pennsylvania CURE Addiction Center of Excellence: Brain Mechanisms of Relapse and Recovery (Childress)
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Title: Amygdalae off the leash ! : Cocaine patients carrying a genetic variant linked to over-response of the stress (cortisol) system show heightened resting connectivity between the left amygdala and the ventromedial prefrontal cortex (vmPFC).

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Abstract: AIM: Our amygdalae are critical first responders in the brain, leaping into action at the first whiff of danger -- or reward. In the healthy brain, these important functions are 'on a leash', tightly regulated by other (inhibitory) brain regions, so that we are not in a chronic state of fear or reward motivation. As stress can undermine the 'leash', we recently tested and confirmed that cocaine patients carrying a genetic variant linked to over- response of the cortisol *stress* system (i.e., the minor G allele of rs3800373, for the FKBP5 gene) have heightened ('unleashed') responding to drug cues in motivational circuits (SfN 2019). Here, we tested whether the vulnerability allele might be linked to heightened functional connectivity between the amygdala and motivational circuitry nodes, *even when the brain is at rest*. **METHODS:** Using BOLD fMRI (3T), we collected six-minute, eyes-open, resting scans in detoxified, treatment-seeking male cocaine inpatients. Resting data were analyzed within the SPM8 connectivity toolbox, generating voxel-wise functional connectivity maps for (R; L) amygdalae in carriers of the minor G (vulnerability) allele (TG, GG; n=15) for rs3800373 of FKBP5, and in TT homozygotes (n=12). The resulting correlation maps (r to Z transformed) were examined for each group, and then formally compared across the two genetic subgroups (group difference t maps thresholded 2-5 for display; FWE- corrected). **RESULTS:** For both cocaine subgroups, there was robust positive "intra-limbic" connectivity between the amygdala (R; L) and multiple motivational nodes (e.g., the ventral tegmental area, ventral pallidum, ventral striatum/putamen, and insula) - and both groups lacked inverse ('leashing') connectivity with the dorsomedial prefrontal cortex (PFC). In addition, carriers of the minor (vulnerability) allele for FKBP5 showed *heightened* resting connectivity between the left amygdala and two motivational nodes, the ventromedial PFC and the insula, as compared to the TT homozygotes. **CONCLUSION**: These new data highlight the 'unleashed' motivational circuitry in cocaine patients, and suggest that carriers of the minor G (vulnerability) allele of rs3800373 for the FKBP5 gene may be at additional risk for psychopathologies related to amygdala over-activity, whether in the domain of aversive (e.g., pain/anxiety/stress/PTSD) or appetitive (e.g., drug reward/relapse) motivation. These genetic findings encourage novel (behavioral, pharmacologic, or neuromodulatory) interventions to boost or to restore regulation of the amygdala, especially for carriers of the vulnerability allele.

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Poster

P726. Addictive Drugs: Human Neural Mechanisms

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Program #/Poster #: P726.03

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01DA048301
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Title: Cortico-striatal volume changes in human cocaine and heroin addiction

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Abstract: While all drugs of abuse share dopaminergic targets, differences in neural morphology between psychostimulant and opiate addiction remain largely unresolved. Compared to opiates, psychostimulant use accompanies increased impulsivity (via inferior frontal gyrus [IFG] and dorsolateral prefrontal cortex [dlPFC]) in humans, and enhanced cue-extinction (via ventromedial PFC [vmPFC]) and neuroplasticity (via nucleus accumbens [NAcc]) in preclinical models. We hypothesized parallel neuroanatomical changes in individuals with cocaine or heroin use disorder (CUD/HUD): specifically, increased NAcc/vmPFC but decreased IFG/dlPFC volumes in CUD vs. HUD. Voxel-based morphometry quantified gray matter volume differences via T1-weighted MRI in demographic/IQ-matched individuals with CUD (n=20; mean age: 42.8 ± 7.3; 5 females), HUD (n=20; mean age: 40.9 ± 10.0; 6 females), and healthy controls (HC; n=20; mean age: 41.4 ± 8.8; 5 females). In line with prior literature, addicted individuals displayed smaller vmPFC volumes than HC ($p<0.05$-corrected)—an effect driven by HUD ($p<0.05$-corrected; similar NAcc compression). Importantly, as hypothesized, there were significant right IFG reductions in CUD vs. HUD ($p<0.05$-corrected); trends for midbrain/NAcc and vmPFC volume increases were further revealed in CUD vs. HUD (uncorrected). Craving was significantly correlated with posterior putamen volume in CUD compared to HUD (the higher the craving, the higher the posterior putamen volume; $p<0.05$ corrected). Consistent with the literature, compared to HC, addicted individuals exhibited mesolimbic cortical/sub-cortical compression. IFG compression and NAcc/vmPFC expansion in CUD vs. HUD extend clinical/preclinical findings, offering for the first time a direct contrast between human CUD and HUD neuroanatomy. The increased posterior putamen volume as a function of craving may be a neural signature of enhanced cue sensitivity in severe CUD. Overall, results suggest neurobiological conservation across species and mechanistic bases for substance-specific neuropsychological differences in humans, with implications for fine-tuning addiction treatment by primary drug of abuse.

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Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: NCCIH Grant R01AT010627

Title: Inter-subject correlations of fMRI responses to cinematic heroin use in individuals with heroin use disorder

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Abstract: Neurophysiological responses to drug cues remain poorly understood in heroin use disorder (HUD). While naturalistic dynamic stimuli (e.g. movies) provide several advantages over traditional fMRI tasks, neural responses to heroin-related naturalistic stimuli have not been studied in HUD. We recorded BOLD activity in 30 treatment-seeking HUD (40.51±9.06 years, 23 M) participants and 16 age, sex, race, and education-matched healthy controls (HC; 43.79±10.33 years, 10 M), while watching the first 17 minutes of the movie "Trainspotting", which contains scenes of explicit heroin use, as well as scenes that are highly salient but not directly related to heroin use. Importantly, the overarching narrative structure of the clip is centered around drug-addiction, including complex contextual elements relevant to HUD (e.g. social, emotional, and economic challenges of addiction). For our analyses, the movie clip was divided into distinct scenes, which were then classified as drug or non-drug, based on whether drugs or speech about drugs appeared in the scene. After watching the movie, participants freely recalled movie details along with relevant personal reflections and rated individual scenes based on their emotional response and heroin craving. To assess neurophysiological responses, we computed whole-brain inter-subject correlation/functional connectivity (ISC/ISFC) of the BOLD signal during the movie, and compared between HUD and HC groups for drug and non-drug scenes. Per the impaired Response Inhibition and Salience Attribution (iRISA) model of drug addiction, whereby excessive incentive salience is ascribed to drug at the expense of non-drug cues/reinforcers, we hypothesize increased ISC during drug-related scenes in the HUD group in reward, salience, habit, executive, self-directed, and memory networks. We also expect ISCs in reward/salience, habit and self-directed networks during drug-related scenes to correlate with craving for those scenes. Similarly, we expect scene-resolved ISCs in the salience and memory networks to correlate with scene recall. Since the movie content should preferentially evoke self-referential thought in the HUD group, we expect these subjects to show elevated ISFC in the default mode network that is time-locked to the explicit drug scenes. This elevated ISFC should predict the duration of self-referential speech when subjects freely recall the movie. Insights

from naturalistic drug cue exposure may better characterize the neural mechanisms of drug cue reactivity in heroin addiction.

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Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant UL1TR003015
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Title: Mindfulness, Future Valuation, and Active Engagement with Integrative Health Practices Support Recovery from Substance Use Disorders

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Abstract: Substance Use Disorders (SUDs) are chronic, remitting, relapsing disorders that are a global public health concern, causing both financial and health burdens for up to 20% of the world population, and requiring long-term treatment. An individual's quality of SUD recovery is multifaceted, including aspects of physical, mental, and social health. However, it is unknown what behavioral determinants underlie success in recovery. We hypothesized that cultivating intentional states of mind, for example through engagement in Integrative Health Practices (IHPs), may be integral in promoting successful recovery. Participants were recruited from the International Quit and Recovery Registry (IQRR), an online research, education, and support platform for individuals who self-report substance misuse. For this study, N=242 individuals meeting the DSM-5 criteria of a lifetime SUD were recruited. Engagement in IHPs was assessed using the International Complementary and Alternative Medicine Questionnaire (I-CAM-Q), whereas state of mind was captured using the Five Facet Mindfulness Questionnaire (FFMQ), Mindful Attention Awareness Scale (MAAS), and the Minute Delay Discounting Task. Quality of recovery was assessed using days in recovery, quality of life, craving, and remission status. We utilized linear and logistic regression modeling to assess whether mindfulness, future valuation, and engagement in IHPs predicted any of our recovery outcomes of interest. Our predictors statistically significantly predicted 1) days in recovery ($F(11, 230)=22.638$, $p<0.001$), accounting for 56% of the variance; 2) quality of life ($F(11, 230)= 26.468$ $p<0.001$), accounting

for 55.9% of the variance; and 3) craving ($F(11, 230)=8.192$, $p<0.001$), accounting for 28.1% of the variance. Additionally, our predictors statistically significantly predicted regression status ($x^2(11)=77.206$, $p<0.001$). Individuals in remission experienced improved quality of life, enhanced future valuation, increased mindfulness, decreased craving, and greater engagement with IHPs. Our results suggest that engaging in IHPs such as meditation, yoga, or tai chi may cultivate states of mind (i.e., mindfulness; future valuation) that promote success in recovery.

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Poster

P726. Addictive Drugs: Human Neural Mechanisms

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Topic: G.09. Drugs of Abuse and Addiction

Support: R21DA034954

Title: Prefrontal-habenular tract abnormalities associated with recent cocaine use in human drug addiction

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Abstract: Drug addiction encompasses neuroadaptations in mesocorticolimbic networks that regulate reward valuation and inhibitory control. The habenula (Hb), an epithalamic hub for communication across cognitive and emotion processing regions, including the prefrontal cortex (PFC), is central to adaptive behaviors that are driven by rewarding or aversive stimuli. Functional impairments in the lateral Hb predict relapse-like behaviors in preclinical models of addiction. However, the Hb's anatomical connections with the PFC, as well as their role in drug addiction, have not been comprehensively studied in humans. Using high-resolution diffusion MRI tractography and diffusion tensor imaging (DTI), we mapped PFC-Hb anatomical connections and examined their white matter microstructure in individuals with cocaine use disorder (CUD; n = 8 female/23 male, mean age = 46 years) and healthy controls (CTL; n = 11 female/17 male, mean age = 42 years). All subjects provided a urine toxicology test for cocaine on the day of scanning, which was used to classify the CUD group into short-term abstinent (CUD-, n = 15) and currently using (CUD+, n = 16) individuals. Tracts were segmented into

component subcortical fiber bundles, the anterior limb of the internal capsule (ALIC) and stria medullaris (SM), and mean DTI metrics were extracted. Analyses of variance (ANOVAs) were performed using a Group × Fiber Bundle × Side design. Two other prefrontal-subcortical white matter projections with trajectories in the ALIC, connecting to the thalamus and midbrain, were similarly examined as a control. The mean fractional anisotropy, a surrogate measure of the coherence of diffusion, showed a significant interaction effect for Group × Fiber Bundle ($F = 6.95$, $p = .001$), with reductions in CUD- vs. CTL along the ALIC ($p = .001$) and in CUD+ vs. CTL along the SM ($p = .018$). There was an additional main effect of Group for the mean diffusivity, which was elevated in CUD- vs. CTL along the entire PFC-Hb tracts ($F = 6.68$, $p = .002$). In the SM, these abnormalities in fractional anisotropy were also significantly correlated with measures of individuals' recency, but not chronicity, of cocaine use ($r = -0.46$, $p = .009$). These effects were specific to the Hb and did not generalize to the adjacent control regions. These results are suggestive of both acute (in the SM) and longer duration (in the ALIC) impairments and support further research into the recovery trajectories of white matter tracts. Importantly, these findings contextualize existing preclinical evidence for PFC-Hb circuit abnormalities in addiction, supporting further investigation into the human Hb and its potential neurodegeneration in addiction.

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Poster

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Title: Parsing motor and stimulus perseveration reveals a novel dissociation between gambling and cocaine use disorders

Authors: *K. ZÜHLSDORFF[1], M. BIRIA[2], J. VERDEJO-ROMÁN[3], L. CLARK[4], N. ALBEIN-URIOS[5], C. SORIANO-MAS[6], T. W. ROBBINS[2], R. N. CARDINAL[2], J. W. DALLEY[2], A. VERDEJO-GARCÍA[7], J. W. KANEN[1];
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Abstract: To flexibly respond to changes in the environment is essential for adaptive behavior in daily life. Flexible decision-making can be modelled using laboratory tests of cognitive flexibility such as probabilistic reversal learning (PRL). Indeed, impairments in cognitive flexibility have been reported in both cocaine and gambling addictions. The mechanistic microstructure of these deficits, however, remains incompletely understood. Reinforcement learning (RL) is the process by which feedback from the environment, such as reward or punishment, is used to adjust behaviour with the aim of maximizing reward and minimizing punishment. RL models have helped gain a more detailed, mechanistic explanation of the latent behavioral processes involved in reversal learning tasks, and how these are altered in various psychiatric disorders. For example, it has been shown that there are decreased learning rates and reinforcement sensitivity (‘value-based’ learning parameters) in depression (Mukherjee et al, 2020), decreased stimulus-bound perseveration (also known as ‘stickiness’, a ‘value-free’ parameter) in obsessive-compulsive disorder, and increased ‘stickiness’ in stimulant use disorder (Kanen et al, 2019). It remains unknown how parameters such as the reward rate, reinforcement sensitivity, location stickiness (motor perseveration) and stimulus stickiness (stimulus-bound perseveration) are differentially affected in cocaine use disorder (CUD) versus gambling addiction and could further fractionate disorders of compulsivity. In the present study, we performed a Bayesian hierarchical reinforcement learning analysis on data from a PRL task conducted on fifty-six participants, which consisted of control subjects (n=18), participants diagnosed with CUD (n=20) or gambling disorder (GD) (n=18). Results demonstrate a reduced reward learning rate in the CUD group and an increase in location stickiness. Strikingly, location stickiness was enhanced in GD, however stimulus stickiness was abnormally low in individuals with GD. Low stimulus stickiness in GD may reflect increased exploratory behavior that is insensitive to outcomes and has been observed in OCD. Alongside the behavioral data, we analyzed task-based fMRI data to relate the observed behavioral findings to their underlying neural mechanisms. Overall, our analysis identified differences between the behavioral processes involved in reversal learning in patients with CUD and GD and brain regions that may be

mediating them. Moreover, we deepen support for RL models providing enhanced specificity for distinguishing perseverative deficits present in different addictive disorders.

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Poster

P726. Addictive Drugs: Human Neural Mechanisms

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P726.08

Topic: G.09. Drugs of Abuse and Addiction

Support: The Menninger Clinic
The Menninger Clinic Foundation
AFSP YIG-1-141-20

Title: Functional connectivity alterations in patients with opioid abuse at high suicide risk

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Abstract: INTRODUCTION: Suicide is a major mental health problem commonly characterized by self-harm and persistent thoughts of wanting to die. Death by suicide is a tragic event that occurs at higher rates in those with psychiatric disorders, including opioid abuse. It remains

unknown whether suicide risk is associated with functional brain connectivity in opioid users. We studied the resting state functional connectivity (RSFC) between the orbitofrontal cortex (OFC) and dorsal striatum (putamen and caudate) in patients at high risk for suicide with and without opioid use. METHODS: Psychiatric patients (N = 75) were recruited from abstinent adult inpatients at The Menninger Clinic (Houston, TX) and divided into groups of opioid-using suicidal patients (OS, N = 35) versus non-opioid-using non-suicidal patients (psychiatric controls: PC, N = 40) based on the Alcohol, Smoking and Substance Involvement Screening Test (ASSIST) for the opioid category and the Columbia-Suicide Severity Rating Scale (C-SSRS) guidelines. OS and PC groups were matched for gender and age. Patients were scanned in a 3T Siemens Trio MR scanner in the Center for Advanced MR Imaging at Baylor College of Medicine. A 4.5 min MPRAGE (1mm isotropic voxels, TE = 2.66ms, TR = 1200ms) was collected, followed by 5 min resting state scan (3.4x3.4x4 mm voxels, TE = 40ms, TR = 2s). RSFC data were preprocessed using the CONN Functional Connectivity Toolbox. The preprocessing pipeline included realignment, slice-timing correction, structural and functional normalization, ART-based outlier detection and smoothing with an 8 mm full width at half maximum Gaussian smoothing kernel. RESULTS: Preliminary data revealed that OS showed significantly higher RSFC between the left medial OFC and left putamen (0.082) than PC (-0.022) ($p < 0.05$). OS also showed higher RSFC between the left medial OFC and right putamen (0.068) than PC (-0.006), but we did not find a significant difference between two groups ($p = 0.1$). RSFC between the left medial OFC and bilateral striatum did not show a significant difference between two groups ($p = 0.9$). CONCLUSIONS: Findings revealed that OS group showed higher functional connectivity between the left medial OFC and left putamen than the PC group. Our data demonstrate that the left medial OFC may be a promising brain target area for brain stimulation techniques for psychiatric patients with high risk of suicide and opioid use. Additional research investigating the potential therapeutic efficacy of brain stimulation (e.g., transcranial magnetic stimulation) to the OFC will be required to provide an effective brain stimulation treatment for patients with suicidality and opioid use.

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Poster

P726. Addictive Drugs: Human Neural Mechanisms

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Program #/Poster #: P726.09

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA
NCCIH

Title: Whole-brain white matter microstructure abnormalities in human cocaine and opiate use disorder

Authors: *P.-O. GAUDREAULT, S. KING, P. MALAKER, N. ALIA-KLEIN, R. Z. GOLDSTEIN;
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Abstract: Functional MRI studies in individuals with substance use disorder commonly show functional connectivity impairments across cortico-striatal circuits that underlie executive control, reward processing and salience attribution. However, substantially few studies have assessed structural connectivity, especially of white matter (WM) tracts, in drug addiction. Our study aimed at using state-of-the-art diffusion MRI analyses to assess whole-brain WM integrity in individuals with cocaine (CUD) or opiate (OUD) use disorder as compared to healthy controls (HC). Diffusion MRI using a 3T scanner was acquired in 58 HC (39.3±8.6yo, 21F), 60 individuals with CUD (current users/CUD+, N=28, 47.7±6.5yo, 6F; and abstinent users/CUD-, N=32, 47.3±7.9yo, 4F) and 30 individuals with OUD (40.3±9.0yo, 6F) on medication-assisted treatment. Diffusion tensors were computed and Tract-Based Spatial Statistics was used for whole-brain/voxel-wise analyses of metrics assessing the coherence of water diffusion along specific orientations [FA-fractional anisotropy, MD-mean diffusivity, AD-axial diffusivity, and RD-radial diffusivity] corrected for age and total intracranial volume. Permutation statistics (p-corrected) were used for group comparisons. Compared to HC, individuals with substance use disorder showed decreased FA and increased AD, MD, and RD ($p<.05$) in all major WM tracts - these effects being primarily driven by CUD+ and OUD. Nevertheless, compared to HC, the CUD- had reduced FA in the left anterior thalamic radiation ($p<.05$). Finally, there was a trend for an increased FA in CUD+ compared to OUD in the forceps major, a posterior WM fiber bundle connecting both occipital lobes through the splenium of the corpus callosum ($p=.05$). Together, these results suggest that individuals with substance use disorder show whole-brain WM impairments defined by less diffusion coherence and directionality as well as increased diffusivity, which are thought to reflect axonal packing attenuation and demyelination. The between-group analyses showing specific whole-brain WM impairments in both groups with positive urine for either cocaine (CUD+) or opiates (OUD) suggest that recency of use might be associated with more severe impact on anatomical connectivity. However, results in the abstinent group (CUD-) also suggest that some WM impairments may persist for longer periods. Further analyses are needed to explore the associations between WM impairments and drug-specific use patterns.

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Poster

P758. Opioids: Reinforcement, Seeking, and Reinstatement V

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Program #/Poster #: P758.01

Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA R01AA027768 (J.W.)
U01AA025932 (J.W.)

Title: Chronic Opioid Administration Potentiates Striatopallidal and Striatonigral GABAergic Transmission

Authors: *W. WANG[1], X. XIE[1], X. ZHUANG[1], T. TAN[1], Z. HUANG[1], H. GANGAL[1], Y. HUANG[1], W. PURVINE[1], X. WANG[1], M. HOOK[1], Y. HUANG[2], J. WANG[1,2];
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Abstract: Opioids such as morphine and fentanyl induce addiction presumably by activating mu-opioid receptors (MORs) in the midbrain and consequently increasing dopamine release in the striatum. The striatum also contains high levels of MORs in spiny projection neurons (SPNs) within the patch compartments. These MOR-positive (MOR+) SPNs project to the internal globus pallidus (GPi) and the substantia nigra pars reticulata (SNr), giving rise to the direct pathway (dSPNs). However, whether chronic opioid exposure alters the activity of MOR+ dSPNs and their outputs remains unclear. Here, we show that MOR activation acutely suppressed inhibitory postsynaptic currents (IPSCs) from dSPNs to habenula-projecting GPi (GPh) neurons through a presynaptic mechanism. Notably, repeated morphine injections potentiated this IPSC or IPSCs from MOR+ SPNs to the GPh. We also discovered that intravenous self-administration (IVSA) of fentanyl enhanced IPSCs from dSPNs to dopamine neurons in the substantia nigra pars compacta (SNc), suppressing dopaminergic activity. Together, these data suggest that chronic opioid use strengthes GABAergic projections from MOR+ dSPNs to both GPh neurons and SNc dopamine neurons, suppressing their activity; such suppression may contribute to the negative emotional states in opioid use disorder.

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Poster

P758. Opioids: Reinforcement, Seeking, and Reinstatement V

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P758.02

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA/NIH

UG3DA050311

Title: Sex differences in the effect of chronic delivery of the buprenorphine analog BU08028 on heroin relapse and choice in a rat model of opioid maintenance

Authors: *L. ALTIDOR[1], A. E. TOWNSEND[2], I. FREDRIKSSON[1], A. SHEKARA[1], S. HUSBANDS[3], A. SULIMA[1], K. C. RICE[1], M. I. BANKS[2], Y. SHAHAM[1], J. M. BOSSERT[1]; [1]IRP/NIDA/NIH, Baltimore, MD; [2]Virginia Commonwealth Univ., Richmond, VA; [3]Univ. of Bath, Bath, United Kingdom

Abstract: Background: Maintenance treatment with opioid agonists (buprenorphine, methadone) decreases opioid use and relapse. We recently modeled maintenance treatment in rats and found that chronic delivery of buprenorphine or the mu opioid receptor (MOR) partial agonist TRV130 decreases relapse to oxycodone seeking and taking. Here, we tested the effect of the buprenorphine analog BU08028 on different heroin relapse-related measures and heroin vs. food choice. **Methods**: For relapse assessment, we trained male and female rats to self-administer heroin (6-h/d, 14-d) in context A and then implanted osmotic minipumps containing BU08028 (0, 0.03, or 0.1 mg/kg/d). We then tested the effect of chronic BU08028 delivery on (1) incubation of heroin seeking in a non-drug context B, (2) extinction responding reinforced by heroin-associated discrete cues in context B, (3) reinstatement of heroin seeking induced by reexposure to context A, and (4) reacquisition of heroin self-administration in context A. For choice assessment, we tested the effect of chronic BU08028 delivery on heroin vs. food choice. **Results:** Chronic BU08028 delivery decreased incubation of heroin seeking in both sexes and extinction responding in males only. Unexpectedly, BU08028 *increased* reacquisition of heroin self-administration selectively in females. Chronic BU08028 had minimal effects on context-induced reinstatement and heroin vs. food choice in both sexes. **Conclusions:** Chronic BU08028 delivery had both beneficial and detrimental sex-dependent effects on different triggers of heroin relapse and had minimal effects on heroin choice in both sexes. Results suggest that BU08028 will not be an effective opioid maintenance treatment in humans.

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Poster

P758. Opioids: Reinforcement, Seeking, and Reinstatement V

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Topic: G.09. Drugs of Abuse and Addiction

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HDRF
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Title: A novel μ-opioid receptor transgenic Cre rat: cellular and behavioral characterization

Authors: *C. MEJIAS-APONTE[1], T. SAUNDERS[2], J. M. BOSSERT[4], L. ALTIDOR[1], M. A. EMERY[3], V. KUMAR[2], A. SEASHOLTZ[2], S. J. WATSON, Jr.[2], J. L. GOMEZ[1], M. MICHAELIDES[5], B. K. HARVEY[6], E. D. HUGHES[2], W. E. FILIPIAK[2], C. RICHIE[1], B. KIEFFER[7], F. VAUTIER[1], H. AKIL[2], Y. SHAHAM[8];
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Abstract: Rats are widely used in animal addiction and pain models. To manipulate μ-opioid receptor-expressing neurons critical to opioid reward and anticonception, we developed a transgenic rat that expresses Cre recombinase under the μ-opioid receptor 1 (*Oprm1*) gene promoter.
We used CRISPR/Cas9 to place T2A self-cleaving peptide and iCre recombinase coding sequences before the termination codon of rat *Oprm1* gene. A long single-stranded DNA donor with short homology arms (98 and 101 nt) flanking the T2a-iCre sequence was synthesized. The donor was mixed with a high specificity sgRNA (5' ACTGCTCCATTGCCCTAACT 3' (PAM = GGG)) and enhanced specificity Cas9 protein prior to Sprague-Dawley (Crl:CD(SD)) rat zygote microinjection. Two founders carrying intact DNA donor in the desired location were identified by DNA sequencing. G0 founders transmitted the correct target T2A-iCre coding sequence to their offspring. For Cre-dependent recombination, we injected a Cre-dependent viral vector into different brain areas to express green fluorescent protein (GFP) in μ-opioid receptor-expressing neurons, identified by *Oprm1* mRNA expression. In initial behavioral characterization, we trained *Oprm1* rats and wild-type littermates to self-administer palatable food. We tested other rats for morphine-induced analgesia and morphine tolerance.
Founder Oprm1-iCre rats selectively expressed P2A-iCre DNA sequence before the stop codon Oprm1 gene. GFP expression was selective to Oprm1-expressing regions: layers 5-6 of mPFC and striatal patches. Dual FISH co-localization studies of iCRE and Oprm1 transcripts in several regions showed that the transgene was exclusively expressed in Oprm1-expressing cells. Food self-administration, morphine-induced analgesia, and morphine tolerance were similar in the transgenic rats and their wild-type littermates.
Oprm1-iCre rats showed selective Cre recombination in brain regions critical to opioid self-administration and relapse. Insertion of Cre under the *Oprm1* gene promoter did not alter palatable food self-administration, morphine-induced analgesia, or morphine tolerance. We introduce a transgenic rat that can be used to gain new insight on the role of μ-opioid receptors-expressing neurons in opioid reward, analgesia, and other behaviors.

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Poster

P758. Opioids: Reinforcement, Seeking, and Reinstatement V

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Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P758.04

Topic: G.09. Drugs of Abuse and Addiction

Support: Supported by NIDA-IRP

Title: Role of ventral subiculum neuronal ensembles in incubation of oxycodone craving after electric barrier-induced voluntary abstinence

Authors: *I. FREDRIKSSON[1], A. BATISTA[1], A. SHEKARA[1], S. V. APPLEBEY[1], A. MINIER-TORIBIO[1], L. ALTIDOR[1], C. CIFANI[2], B. T. HOPE[1], J. M. BOSSERT[1], Y. SHAHAM[1];
[1]NIH, NIDA, IRP, Baltimore, MD; [2]Univ. of Camerino, Sch. of Pharm., Camerino, Italy

Abstract: Background: We recently developed a rat model of incubation of oxycodone craving after voluntary suppression of drug self-administration by adverse consequences of drug seeking. Here, we studied the role of ventral subiculum (vSub) neuronal ensembles in this incubation, using the activity marker Fos, muscimol-baclofen (GABAergic agonists) inactivation, and Daun02 chemogenetic inactivation. **Methods:** We trained Sprague-Dawley or *Fos-lacZ* transgenic male and female rats to self-administer oxycodone (0.1 mg/kg/infusion, 6-h/d) for 14 days. The rats were then exposed for 14 days to an electric barrier of increasing intensity (0.1 to 0.4 mA) near the drug-paired lever that caused voluntary abstinence or were exposed to 14 days of forced abstinence. We tested Sprague-Dawley rats for relapse to oxycodone seeking without shock and drug on abstinence day 15 and extracted their brains for Fos-immunohistochemistry, or tested them after vSub vehicle or muscimol-baclofen injections on abstinence days 1 and 15. We performed Daun02 inactivation of relapse-activated vSub Fos neurons in Fos-lacZ transgenic rats on abstinence day 15 and then tested them for relapse on abstinence day 18. **Results:** Relapse after electric barrier-induced abstinence increased Fos expression in vSub. Muscimol-baclofen inactivation or Daun02 selective inactivation of vSub Fos-expressing neuronal ensembles decreased “incubated” oxycodone seeking after voluntary abstinence. Muscimol-baclofen vSub inactivation had no effect on non-incubated opioid seeking on abstinence day 1 or incubation after forced abstinence. **Conclusions:** Results demonstrate a selective role of vSub neuronal ensembles in incubation of opioid craving after cessation of drug self-administration by adverse consequences of drug seeking.

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Poster

P758. Opioids: Reinforcement, Seeking, and Reinstatement V

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Program #/Poster #: P758.05

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA/NIH

Title: Choice between social interaction and remifentanil in rats

Authors: *J. J. CHOW[1], J. M. CHABOT[1], M. VENNIRO[2], Y. SHAHAM[1];
[1]IRP/NIDA/NIH, Baltimore, MD; [2]Univ. of Maryland, Baltimore, MD

Abstract: Background: Clinical and preclinical studies have demonstrated that the availability of non-drug rewards can decrease choice for opioids. In humans, non-drug alternatives are primarily social in nature. Addressing this preclinical-clinical gap, we developed a rat model of operant social self-administration and choice between social interaction and drugs. Here we examined how access duration to social interaction and remifentanil dose affects choice. **Methods:** We trained rats (n=16; 8 females) to lever-press for social interaction and tested different durations of access (3.75s to 240s). We then assessed if these different durations influenced the rewarding aspect of social interaction, using progressive ratio and choice procedures. Next, we trained another cohort of rats (n=16; 7 females) to lever-press for 15s social interaction. Following IV-catheterization, we trained the rats to self-administer 10 µg/kg/infusion remifentanil. We trained the rats on a controlled reinforcer frequency choice procedure and conducted discrete-trials probe-tests, comparing choice between 15s of social interaction and remifentanil (0, 1.0, and 10 µg/kg/infusion). **Results:** We observed a decrease in social rewards earned as access duration increased. However, progressive ratio and choice measures revealed that rats were not sensitive to access duration. Rats trained for social interaction and remifentanil choice demonstrated preference for remifentanil at 10 µg/kg/infusion, indifference at 1.0 µg/kg/infusion, and preference for social interaction at 0 µg/kg/infusion. **Conclusions:** Rats' insensitivity to access duration allows the use of shorter durations of social interaction, optimal for time-sensitive in vivo recording measures. Choice between remifentanil and social interaction was dose-dependent, proving useful for the development of our choice model and investigation into neurobiological mechanisms of preference for social interaction vs. opioid drugs.

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Poster

P758. Opioids: Reinforcement, Seeking, and Reinstatement V

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Topic: G.09. Drugs of Abuse and Addiction

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Title: Role of piriform cortex afferent projections in relapse to fentanyl seeking after food choice-induced voluntary abstinence

Authors: *D. J. REINER[1], S. M. CLAYPOOL[1], S. BEHDIN[1], J. ORIHUEL[1], Y. SHAHAM[2]; [1]IRP-NIDA, NIH, Baltimore, MD; [2]IRP/NIDA/NIH, Baltimore, MD

Abstract: Background: We previously showed a role of piriform cortex (Pir) in relapse to fentanyl seeking after food choice-induced voluntary abstinence, a procedure that mimics abstinence due to availability of alternative non-drug rewards. Here, we used retrograde tracing to determine projection-specific activation of Pir afferent projections during fentanyl relapse by using Fos plus the retrograde tracer cholera toxin B (injected into Pir). **Methods:** We trained male and female rats to self-administer palatable food pellets for 6 days (6-h/day) and fentanyl (2.5 microgram/kg/infusion, i.v.) for 12 days (6-h/day). We assessed relapse to fentanyl seeking after 14 voluntary abstinence days, achieved through a discrete choice procedure between fentanyl and palatable food (20 trials/day). **Results:** Relapse to fentanyl seeking was associated with increased Fos expression in neurons in anterior insular (AI) and prelimbic (PL) cortex that project to Pir but not Pir-projecting cortical neurons in adjacent areas or Pir-projecting thalamic neurons. Preliminary anterograde tracing in AI or PL confirmed these AI-to-Pir and PL-to-Pir projections. **Conclusions:** Results demonstrate a correlational role of AI-to-Pir and PL-to-Pir projections in relapse to fentanyl seeking after food choice-induced abstinence. In future experiments, we will determine the causal role of these projections in relapse to fentanyl seeking after food choice-induced abstinence.

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Poster

P758. Opioids: Reinforcement, Seeking, and Reinstatement V

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Topic: G.09. Drugs of Abuse and Addiction

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Funds to NIDA IRP
P30DA0333034

Title: Lack of effect of different pain-related manipulations on opioid self-administration, reinstatement of opioid seeking, and opioid choice in rats

Authors: *S. CLAYPOOL[1], E. TOWNSEND[2], J. ORIHUEL[1], S. V. APPLEBEY[1], M. BANKS[2], Y. SHAHAM[1], S. NEGUS[2], D. J. REINER[1];
[1]Natl. Inst. on Drug Abuse, Baltimore, MD; [2]Virginia Commonwealth Univ., Richmond, VA

Abstract: Rationale and Objective: Pain-related factors increase risk for opioid addiction, and pain may function as a negative reinforcer to increase opioid taking and seeking. However, experimental pain-related manipulations generally do not increase opioid self-administration in rodents. This discrepancy may reflect insufficient learning of pain-relief contingencies or confounding effects of pain-related behavioral impairments. Here we determined if pairing noxious stimuli with opioid self-administration would promote pain-related reinstatement of opioid seeking or increase opioid choice over food.
Methods: In Experiment 1, rats self-administered fentanyl in the presence or absence of repeated intraplantar capsaicin injections in distinct contexts to model context-specific exposure to cutaneous nociception. After capsaicin-free extinction in both contexts, we tested if capsaicin would reinstate fentanyl seeking. In Experiment 2, rats self-administered heroin after intraperitoneal (i.p.) lactic acid injections to model acute visceral inflammatory pain. After lactic acid-free extinction, we tested if lactic acid would reinstate heroin seeking. In Experiment 3, we tested if repeated i.p. lactic acid or intraplantar Complete Freund's Adjuvant (CFA; to model sustained inflammatory pain) would increase fentanyl choice over food.
Results: In Experiments 1-2, neither capsaicin nor lactic acid reinstated opioid seeking after extinction, and lactic acid did not increase heroin-induced reinstatement. In Experiment 3, lactic acid and CFA decreased reinforcement rate without affecting fentanyl choice.
Conclusions: Results extend the range of conditions across which pain-related manipulations fail to increase opioid seeking in rats and suggest that enhanced opioid-addiction risk in humans with chronic pain involves factors other than enhanced opioid reinforcement and relapse.

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Poster

P783. Brain Mechanisms of Learning and Decision-Making

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Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P783.01

Topic: H.03. Decision Making

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Title: Circuit-level mechanism for adjustments of learning to uncertainty in reward environment

Authors: *S. FARASHAHI[1], A. SOLTANI[2];
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Abstract: Successful learning involves integration of reward outcomes over time. However, determining the time constant of this integration is non-trivial when reward is probabilistic and reward contingencies can unpredictably change over time, as in naturalistic settings. Fitting of choice behavior using reinforcement learning or more mechanistic models of synaptic plasticity suggests that the learning rates, which determine the time constant of reward integration, are adjusted according to uncertainty and volatility of the reward environment. However, it is currently unknown how such adjustments can be instantiated by circuit-level interactions.To answer this question, we constructed biologically-inspired recurrent networks of excitatory and inhibitory point neurons endowed with plausible reward-dependent Hebbian learning rules. We trained these recurrent neural networks (RNNs) to perform a probabilistic reversal learning (PRL) task with different reward probabilities (controlling uncertainty) and reversal length (controlling volatility or frequency of changes in reward contingencies).We found that the trained RNNs can robustly perform the PRL task by dynamically adjusting their learning according to uncertainty and volatility. Analyses of neural response and connectivity in RNNs revealed that adjustments of learning to uncertainty and volatility relied on distinct synaptic currents in the circuit. More specifically, the adjustment of learning rate to uncertainty was accompanied by modulations in the strength of synaptic currents from inhibitory to excitatory units. In contrast, the adjustment of learning rate to volatility was accompanied by modulations of synaptic currents between excitatory units. Analysis of lesioned neural networks confirmed the causal role of these currents in adjustments of the learning rate and were compatible with our correlation analysis. Together, our results provide a plausible circuit-level mechanism for adjustments of learning to different types of uncertainty, and moreover, provide testable predications about distinct roles of inhibitory and excitatory connections in adaptive behavior.

Disclosures: S. Farashahi: None. **A. Soltani:** None.

Poster

P783. Brain Mechanisms of Learning and Decision-Making

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P783.02

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NIH R01 DC018545
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Title: History integration across diverse temporal horizons in retrosplenial cortex may give rise to hyperbolic-like behavior

Authors: *B. P. DANSKIN, Y. E. ZHANG, R. HATTORI, M. C. AOI, T. KOMIYAMA; Univ. of California San Diego, La Jolla, CA

Abstract: Animals integrate history information to guide future decisions, particularly in a dynamic environment. Reinforcement learning (RL) theory provides a model to estimate integrated action values for decision making. However, this estimation is insufficient to capture certain aspects of decision-making. Specifically, standard RL models (e.g. Rescorla-Wagner model) fail to capture heavy-tailed integration—the temporal decay of near and distant past experience at different rates—that is common feature of decision-making in dynamic environments. Here we explore an alternative model where decision is reached by multiple independent integrators that update their expected value across a spectrum of learning rates. We trained mice to perform a foraging task in which the reward probability changes dynamically and unpredictably. The mice integrate information about past choice and reward to estimate the difference in value of the two options to guide their decision. The pattern of history weighting by mice showed a heavy-tail that was better approximated by hyperbolic than exponential integration. To explore the neural basis of such behavioral integration, we analyzed neural population activity recorded from six cortical areas during this behavioral task. Individual history-encoding neurons integrate reward-choice history with a variety of temporal horizons, and the discounting patterns were closer to exponential than hyperbolic, in contrast to behavior. In the retrosplenial cortex (RSC), the distribution of time constants of history integration across neurons was particularly skewed to longer time constants compared to the other five areas. The summation of these diverse integration patterns across RSC neurons reproduced the hyperbolic-like behavioral integration in mice. Our results suggest that value maintained separately across neurons yields the heavy-tailed integration observed in the behavior, and identify RSC as a repository of this diverse temporal information.

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Poster

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Support: National Science Foundation grant DMS-1608077 (HGR)

Title: Resonance-based flexible selection of cognitive tasks and memory suppression in a hippocampus-prefrontal cortex network regulated by the nucleus reuniens

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Abstract: Our ability to switch and perform an action in response to attended information is known as cognitive flexibility. The prefrontal cortex (PFC) is responsible for selecting and flexible routing oscillatory items from the hippocampus (HPC) to target areas. These may receive commands from PFC to suppress items in HPC. Recently, a PFC modeling study showed that multiple stored items could be selected by making use of firing rate resonance (f_r) and lateral inhibition (Sherfey et al., 2020). However, there is evidence that PFC and HPC transient coupling via oscillatory-synchrony is favored and switches due to the nucleus reuniens (Re). The mechanisms governing how these structures cooperatively operate are not well understood. We develop a PFC-HPC-Re model having principal cells (PCs) and interneurons (INs). For the PFC, two PC populations connected through a lateral IN population represent gates to downstream activity; For the HPC, local INs are connected preferentially to closer PCs which receive square-wave Poisson modulated spikes with different frequencies generating oscillatory activity; the Re provides excitatory input to both HPC and PFC with variable degree of excitation. This extends previous models by using (i) simpler neurons, facilitating the mechanistic understanding of the routing, (ii) a HPC network where memory is stored prior to PFC activation, and (iii) the effect of Re, which provides additional network flexibility. Gate PCs receiving an input frequency from HPC closer to f_r will fire more and engage with the IN population suppressing other PCs. Only firing rate resonance and inhibition suffice for this PFC effect as showed before. We show that the PFC chosen inputs (given its proximity with the gate's internal f_r,) can be switched by the Re activity, which alters the selected item periodicity. In addition, Re input into PFC can awaken an otherwise suppressed gate and engage with HPC reversing the direction flow. Thus Re provides a wider range of network flexibility influencing the cognitive selection and memory suppression. A reduced rate model reproduces the same pattern of choices and suppression when Re input is provided. Our results demonstrate the importance of Re in routing oscillatory-synchrony HPC-PFC in both directions confirming previous experimental observations. HPC local inhibition maintains many stored items in the same network agreeing with recent observations, which suggest that INs allow memory separation by their connectivity pattern. Re allows flexible

control of the HPC-PFC creating competition between PFC resonant networks in cognitive selection and HPC memory storage through activation of local inhibition.

Disclosures: R.F.O. Pena: None. **H.G. Rotstein:** None.

Poster

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Program #/Poster #: P783.04

Topic: H.03. Decision Making

Title: The geometry of neural representations can predict significant but unexpected behavioral differences in two monkeys

Authors: *V. FASCIANELLI[1,2], F. STEFANINI[1,2,3], S. TSUJIMOTO[4], A. GENOVESIO[5], S. FUSI[1,2,6,7];
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Abstract: Our ability to perform cognitive tasks depends on the geometry of neural representations. High dimensional representations allow a downstream linear readout, like a neuron, to implement a large number of I/O functions. In contrast, low dimensional representations, which typically require a process of abstraction, allow for better generalization, but they restrict the I/O functions implementable by a linear readout. An interesting compromise is to dynamically adjust the geometry depending on task demands. Using analytical tools developed to study abstraction, we observed that the geometry of the neural representations can change rapidly during the trial and, importantly, it can explain different behaviors.
We studied the activity of dorsolateral prefrontal cortex in two monkeys performing a visually cued strategy task. Two visual stimuli of different shapes and colors cued the repetition of the previous response (stay strategy), whereas other two visual stimuli cued to shift to the alternative response (shift strategy). Most relevant task variables are decoded with high accuracy in both monkeys, including the visual cue and the strategy. However, only a few were in an abstract format. In particular, during the cue presentation, the first monkey represents the visual cue in an abstract format, while the second represents the strategy in an abstract format, as indicated by the Cross Condition Generalization Performance (the performance of a linear decoder able to generalize to task conditions not used for training). The first monkey shows a reaction time (RT) that depends on the cue's shape, regardless of the strategy. While the second, shows a RT depending on the strategy, regardless of the shape. To address this difference, we trained a

feedforward neural network per monkey to perform the task. The main difference in the two models is the presence of an autoencoder in the output layer forcing one network to reproduce the visual cue, and the other to reproduce the previous response. We succeeded in reproducing the neuronal representations of task variables along with different dynamics, mimicking the RTs of both monkeys.
Our analysis reveals interesting differences in neuronal representations in two monkeys performing the same task with high performance. This difference is mirrored by the different geometry of the neural representation of two task variables: the visual cue and strategy. Interestingly, the geometry can predict a difference in RT that we were not aware of, which we discovered by reanalyzing the details of monkeys' behavior. This relation between geometry and behavior is explained by a simple model.

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Poster

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Program #/Poster #: P783.05

Topic: H.03. Decision Making

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R01MH108629

Title: Effects of volatility on prefrontal neural dynamics and possible underlying neural mechanisms

Authors: *M. MORADI SPITMAAN[1], C. H. DONAHUE[3], D. LEE[4], A. SOLTANI[2]; [2]Psychological & Brain Sci., [1]Dartmouth Col., Hanover, NH; [3]Neurosci., The Gladstone Inst., San Francisco, CA; [4]Neurosci., Yale Sch. of Med., New Haven, CT

Abstract: Activity of cortical neurons exhibits multiple dynamics related to the integration of task-relevant signals such as choice and reward outcomes. In addition, the time constant of reward integration is correlated with the time constant of behavioral adjustments to reward, often measured by the learning rate in reinforcement learning (RL) models. Although there is growing behavioral evidence for adjustments of learning to volatility in the reward environment, it is currently unknown if different dynamics of neural response are modulated by volatility and if so, how such modulations are instantiated in the brain. To address these questions, we analyzed single-cell recordings in three cortical areas (dlPFC, OFC, and ACC) during a probabilistic reversal learning task involving reward probability and magnitude, and under two levels of volatility. Reward magnitudes on two alternative options were presented on each trial while

reward probabilities, which must be learned through reward feedback, remained unchanged or underwent reversals in the stable and volatile conditions, respectively. Moreover, we used two different learning models based on plastic and metaplastic synapses to study the encoding and decoding of reward information in an ensemble of estimators with different levels of plasticity. We found evidence for the effects of volatility on the integration of reward outcome (reward memory) and identity of the chosen stimulus (stimulus memory). Specifically, reward-memory timescales were larger in the volatile than the stable condition in both OFC and ACC. In contrast, stimulus-memory timescales were smaller in the stable condition but only for dlPFC neurons. Moreover, the variance of both reward- and stimulus-memory amplitudes were significantly larger in the volatile condition in all cortical areas. These suggest that volatility could expand the heterogeneity in the integration of task-relevant signals by modulating memory timescales and diversifying memory amplitudes to allow better estimation of task-relevant signals in volatile environments.We next used the two computational models to explain the link between such modulatory processes and the involvement of neurons in integrating reward information. We found that the metaplastic model showed higher performance in estimating reward probability by creating more heterogenous estimates across the ensemble. Together, our results suggest that metaplasticity can create a heterogeneous ensemble of reward estimators similar to a diverse ensemble of reward-integrating neurons, and this allows for better decoding of reward in volatile environments.

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Poster

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Title: Discovering flexible models of neural population dynamics underlying decision-making

Authors: *M. GENKIN[1], C. CHANDRASEKARAN[2], K. V. SHENOY[3], T. A. ENGEL[1]; [1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [2]Psychological & Brain Sci, Anat. & Neurobiology, Ctr. for Systems Neuroscience, Biomed. Eng, Boston Univ., Boston, MA; [3]EE, BioE, Neurobiology, Wu Tsai Neurosci. Inst. & Bio-X, Stanford Univ. & Howard Hughes Med. Inst., Stanford, CA

Abstract: Behaviorally relevant signals are often represented in neural population dynamics, which evolve on a low-dimensional manifold embedded into a high-dimensional space of neural responses. Revealing population dynamics from spikes is challenging because the dynamics and embedding are nonlinear and obscured by diverse responses of individual neurons and spiking noise. For example, the decision-related activity of single neurons was hypothesized to arise from either gradual ramping or abrupt stepping dynamics on single trials, but selection between these alternatives is brittle due to the diversity of neural responses. Moreover, ramping and stepping are impoverished hypotheses for heterogeneous decision-related neural populations. We need frameworks that can flexibly identify neural population dynamics from data.
We developed a flexible framework for inferring neural population dynamics from spikes. In our framework, population dynamics are modeled by a latent nonlinear dynamical system defined by a potential function. The framework covers a continuous space of hypotheses represented by different potential shapes. The activity of individual neurons is related to the population dynamics through unique firing-rate functions, which account for the heterogeneity of neural responses. We incorporate the distribution of the population state at the trial start and boundary conditions modeling trial termination, such as commitment to a choice when reaching a decision boundary. The potential, firing-rate functions, and initial state distribution are continuous functions that are inferred from data using gradient-descent optimization.
On simulated neurons, our framework correctly recovered the ramping and stepping models, which correspond to linear and three-well potentials, respectively. We applied the framework to neural activity recorded from the macaque dorsal premotor cortex (PMd) during a decision-making task. The inferred potential revealed dynamics that evolve gradually towards the correct choice but have to overcome a potential barrier towards the incorrect choice. We obtained the same results by fitting multiple neurons simultaneously or each neuron separately, and the results were robust across neurons. Our results are consistent with the dynamics generated by attractor models of decision-making (e.g., Wang 2002) and differ from ramping and stepping hypotheses proposed previously. Our study demonstrates that a flexible approach can discover new hypotheses about decision-related population dynamics from spiking activity.

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Poster

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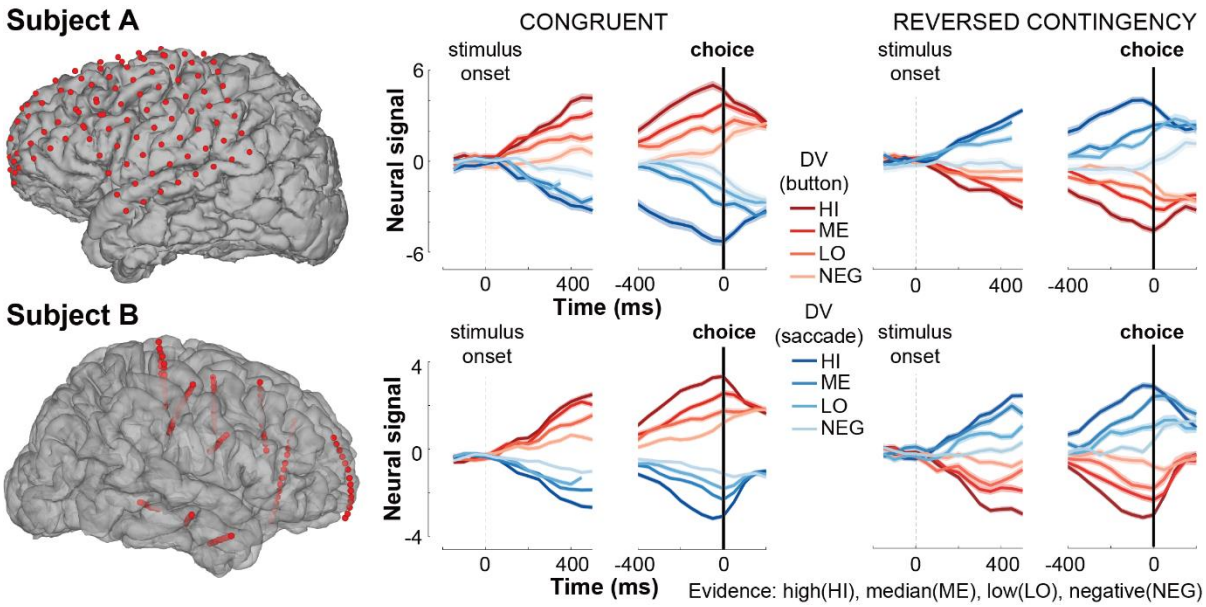
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Title: Decision dynamics in the brain

Authors: *T. XIE[1,3], M. ADAMEK[2,3], P. BRUNNER[1,3,4], J. KUBANEK[5];
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Abstract: Decisions often involved accumulating evidence over time, and this process has commonly been characterized using diffusion decision models (DDMs). In this class of models, subjects accumulate decision evidence up to a certain fixed bound, at which time they make a choice. We tested how DDMs are supported by neural data recorded in human subjects. We recorded cortical/subcortical neural signals in 8 humans engaged in decisions that required integration of individual quanta of evidence. The subjects communicated their choice using eye or hand movements. We recorded data in two versions of the task. In the fixed duration (FD) task, the time to make a choice was cued; in the reaction time (RT) task, subjects could indicate their choice at any time. We first inferred a decision variable (DV) for this task according to the Signal Detection Theory. The DV faithfully captured the subjects' choices and reaction times. We then identified the neural signals that encode the DV. In particular, we regressed signal features in specific frequency bands, using signals from all channels, on the DV. This provided us with the neural signal (NS) representing the diffusion process in this task. In line with the DDMs, the NS represented the accumulated evidence. The drift rate was proportional to the DV. As expected, this ramping activity was weak in the error trials. Single-trial analysis revealed that the ramps were real, not a consequence of averaging discrete jumps. Moreover, in accordance with the DDMs, the NS reached a fixed bound in the FD task. Intriguingly, the neural signal did not reach a fixed bound in the RT task. In this task, the NS remained strongly graded by the DV at the time of the subjects' choice (examples of this phenomenon are shown for two subjects in the figure). This prominent finding is in odds with the common formulations of the DDM and also in odds with the signals reported in monkey area LIP, which show a fixed bound in RT tasks. Our results suggest that classic formulations of the DDMs may need to be expanded to accommodate bounds of varying levels, flexible bounds, or revisit the concept of a bound entirely.



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Poster

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Support: NIH U19 Grant

Title: Choice-selective sequences dominate in cortical relative to thalamic inputs to nucleus accumbens, providing a potential substrate for credit assignment.

Authors: *A. BAIDYA[1], N. F. PARKER[2], J. M. COX[3], M. S. GOLDMAN[1], I. B. WITTEN[4];
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[3]Northwestern Univ., Evanston, IL; [4]Princeton Univ., Princeton, NJ

Abstract: A central question in reinforcement learning is how actions and outcomes separated in time become associated with each other. Multiple lines of evidence have implicated the nucleus accumbens, a part of the ventral striatum that receives cortical and thalamic inputs as well as dopaminergic signals associated with reward prediction errors, in learning action-outcome associations. Here we image the activity of cortical and thalamic neurons projecting directly to the nucleus accumbens during a two-armed bandit task in which an animal must determine the

lever corresponding to the higher probability of reward. Prelimbic cortical inputs preferentially encoded animals' recent actions (choices) on a given trial, whereas thalamic neurons preferentially encoded external cues (stimuli). The cortical input population exhibited choice-selective sequences of neural firing that bridged a delay period between action (choice) and outcome (reward). Through computational modeling, we demonstrate how such choice-selective sequences can create the precisely timed reward prediction error seen in dopamine neurons which, in turn, can support the neural implementation of reinforcement learning algorithms for associating actions and outcomes separated in time, both in a circuit model based on dopamine-dependent changes in synaptic plasticity and one based on dopamine-dependent changes in neural dynamics. By optogenetically manipulating both the cortical and thalamic input neurons we tested and confirmed core predictions of both the circuit models. Thus, by recording and manipulating glutamatergic inputs to the NAc and integrating these data with computational modeling, we provide specific proposals for how reinforcement learning could be implemented by neural circuitry and suggest a computational role for choice-selective sequences of neural activity.

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Poster

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Support: UNM Grand Challenge

Title: Neurocomputational markers of cue-specific exploration in alcohol use disorder

Authors: *E. M. CAMPBELL[1], G. SINGH[1], E. D. CLAUS[2], K. A. WITKIEWITZ[1], V. D. COSTA[3], J. HOGEVEEN[1], J. F. CAVANAGH[1];
[1]Univ. of New Mexico, Albuquerque, NM; [2]Mind Res. Network, Albuquerque, NM; [3]Dept. of Behavioral Neurosci., OHSU/ONPRC, Portland, OR

Abstract: Humans often face the difficult decision between exploring novel options with uncertain outcomes, versus exploiting familiar options with a known reward value. Prior work has linked hypersensitivity to familiar rewards to alcohol use disorder (AUD), but the neural computations mediating the drive to explore novel alcohol cues in individuals with AUD remain unclear. We merged EEG and computational modelling of a probabilistic reinforcement learning task to assay the neurocomputational bases of novelty-driven exploration in AUD. Individuals with AUD (n = 28) and matched controls (n = 27) completed a three-armed bandit task with

images of alcoholic beverages and non-alcoholic beverages, which were periodically replaced by novel stimuli with unknown reward values. Behavior and computational modelling results converged to demonstrate heightened cue-specific exploration of alcohol stimuli in individuals with AUD. This finding was associated with aberrant P3a responses in AUD, which scaled with enhanced cue-specific exploratory behavior suggesting that changes in functional cortical dynamics characterize stimulus-dependent exploration in AUD.

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Poster

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Title: A neural representation of probabilistic belief in human posterior parietal cortex

Authors: *N. M. SINGLETARY[1], J. GOTTLIEB[1], G. HORGA[2];
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Abstract: Bayesian theory states that optimal probabilistic beliefs require the integration of new evidence with prior beliefs in proportion to the reliability of each factor. However, human neuroimaging studies have incompletely dissociated neural responses to posterior probability, prior probability, and likelihood (the reliability of new information), making it difficult to elucidate the neural mechanisms by which humans update their beliefs. In addition, the studies have not controlled for expected value (EV), which is correlated with posterior probability when participants make instrumental decisions. To examine these questions, we developed a probabilistic inference task in which human participants (N = 23, 9 female, age: 19-64 years) estimated the posterior probability of being in one of two hidden states: a "portrait gallery" containing more pictures of faces than scenes or a "landscape gallery" containing more pictures of scenes than faces. We independently manipulated the prior probability of being in each type of gallery (7-93%), the type of new information (a face or landscape picture randomly drawn from the true gallery), and the diagnosticity of the information (an indicator of the likelihood) to identify the neural correlates of each factor. Moreover, we used an incentive-compatible procedure to elicit posterior estimates and varied the penalty for an incorrect estimate ($10 or $20 subtracted from a $30 endowment) to control for EV. Analysis of behavior showed that

participants incorporated prior probability and likelihood into their posterior probability estimates but were not influenced by the size of the penalty. Analysis of fMRI blood oxygen level-dependent (BOLD) activation identified two regions of the left superior parietal lobule (SPL) and intraparietal sulcus (IPS) that showed significant correlation with posterior probability estimates and were also positively correlated with both prior probability and likelihood. Activation in these regions could not be explained by eye or hand movements or, crucially, by EV. Thus, we propose that human SPL and IPS represent probabilistic posterior beliefs and may be neural substrates for approximating Bayesian inference.

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Poster

P876. Entorhinal Cortex: Relational and Spatial Learning

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Topic: H.10. Human Learning and Cognition

Support: ONR N00014-20-1-2578
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Title: Hippocampal sharp-wave ripples and cortico-hippocampal interactions during goal-directed navigation

Authors: *K. KIM[1], C. RANGANATH, Ph.D.[1], J. A. OVERTON[1], R. S. DHANAK[1], F. GIRGIS[2], K. SHAHLAIE[1], J. LIN[3], R. C. O'REILLY[1], I. SAEZ[1];
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Abstract: Hippocampal sharp-wave ripples (SWRs) reflect synchronized bouts of neuronal firing. Research in rodents suggests that SWRs are associated with rapid memory replays (Jadhav et al., 2012; Roumis & Frank, 2015; Roux et al., 2017) and interactions with the prefrontal cortex (Tang & Jadhav, 2019) during memory-guided decision making and spatial learning. Much less is understood about SWRs in humans, but recent work suggests that awake hippocampal SWRs in the human brain may also play an essential role in online cognitive behaviors such as memory retrieval (Norman et al., 2019). In the present study, we investigated the role of the awake hippocampal SWRs during goal-directed spatial navigation and learning, as well as cortico-hippocampal interactions during SWR events. Specifically, we hypothesized that hippocampal SWRs would increase as a function of learning, especially prior to events that involve rapid retrieval and evaluation of prior experiences - such as decision responses and goal expectations. To test this, we recorded intracranial electroencephalography (iEEG) from human patients (N = 6) while they were engaged in a navigation task that has a learning component.

Patients controlled an agent to navigate through 2D mazes and reach a hidden goal (i.e., exit) position as efficiently - with fewest number of moves - as possible. Importantly, paths in the mazes were only partially visible and were revealed along the routes the agent took, and thereby the goal location was not visible until the agent reached a near-goal location. The task included 30 unique mazes, which were repeated across 2 blocks. We analyzed iEEG data from hippocampal and orbitofrontal cortex (OFC) electrodes. We found that SWR rates were greater in the second block compared to the first block at the critical events of interest: prior to decision responses and prior to goal revelation. Strong broad-band oscillatory activity emerged in the OFC during hippocampal SWRs. The present results suggest that hippocampal SWRs may drive memory replay during memory-guided, goal-directed behaviors in the human brain.

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Poster

P876. Entorhinal Cortex: Relational and Spatial Learning

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Topic: H.10. Human Learning and Cognition

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Title: A neurobiological model of entorhinal cortex and hippocampus to explain forgetting and spacing effects

Authors: *J. ANTONY[1], X. LIU[2], C. RANGANATH, Ph.D.[3], R. O'REILLY[1];
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Abstract: Episodic memory involves binding various pieces of information to a particular context and relies on the hippocampus (HC). Recent work suggests that the entorhinal cortex (EC), one major input to the HC, may play a crucial role in representing information about temporal context. Specifically, EC neurons “drift”, or change slowly across a spectrum of time scales, from seconds to hours. Intriguingly, this drifting temporal representation may constitute an episodic memory cue itself, becoming weaker over time and explaining phenomena such as forgetting curves. It could also help explain the robust, time-varying phenomenon known as the spacing effect, i.e. how re-learning after longer (versus shorter) delays improves long-term memory. Here, we advanced a neurobiologically realistic model of the EC and HC with time-drifting properties that accounts for forgetting and the spacing effect. Learning in our model is driven by error, or differences between stored and current patterns, which also drives learning in the brain (O’Reilly et al., 2021). We predicted that spacing effects arise because longer delays produce larger differences between the stored and current temporal contexts in EC, which results

in greater error and stronger HC learning. We tested memory performance in the model on a paired associate task, whereby the model learned to relate two random input vectors along with the concurrent temporal context. We simulated temporal drift as input vectors into EC that gradually changed across a spectrum of timescales. We then tested the ability of the model to reproduce the target associate while varying temporal delays (drift) between successive learning episodes and/or for the final test. First, we found that greater delays after learning in the model reproduced forgetting curves. Next, we tested model performance after different delays between learning episodes. We found that increasing delays produced both greater error in HC area CA3 and better subsequent long-term memory performance. We also tested learning across logarithmically expanding intervals - a commonly studied learning technique - against contracting intervals. In accord with prior behavioral work, expanding drift helped memory after long delays while contracting drift helped after short delays. Additionally, representational activation patterns in CA3 across rounds of learning showed that, with greater drift, the model learned by reducing its reliance on short time scale patterns, effectively generalizing over time. Altogether, these results could provide a mechanistic formalization for established learning concepts such the importance of varying contexts and errors during learning.

Disclosures: J. Antony: None. **X. Liu:** None. **C. Ranganath:** None. **R. O'Reilly:** A. Employment/Salary (full or part-time):; eCortex.

Poster

P876. Entorhinal Cortex: Relational and Spatial Learning

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:30 PM - 2:30 PM

Program #/Poster #: P876.03

Topic: H.10. Human Learning and Cognition

Support: ONR N00014-17-1-2961
NSF 2022685

Title: Representations of predictive relations in entorhinal cortex and middle temporal gyrus as a function of exposure and time

Authors: *A. LESHINSKAYA, M. A. NGUYEN, C. RANGANATH, Ph.D.;
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Abstract: The ability to learn predictive relations among events (such as thunder preceding rain) is an essential aspect of adaptive behavior. Anterior-lateral entorhinal cortex (alERC) is critical for the acquisition and initial recall of predictive relations from experience, but there is reason to believe that neural representations of such relations might change or become reorganized over time. To investigate such changes, we used fMRI to measure neural representations of predictive relations between events both immediately after learning (Session 1) and after a one week delay

and additional exposure (Session 2). Twenty-four human participants were scanned as they watched 6 movies, each depicting a distinct object embedded in a sequence of animated visual events. In each sequence, one event A strongly predicted another event B, while events C - F were unpredictable. Among Consistent sequences, the same event served as predictable event B, whereas among Inconsistent sequences, the predictable event B was swapped with an event that was unpredictable in the other sequences. After each movie, participants saw a Probe phase, in which they saw the same events in discontinuous, randomized order. To identify neural representations of predictive relations, we subtracted voxel pattern similarity between predictive events pairs A & B vs that between unpredictive event pairs such as A & C ("Associative Coding"). The same protocol was repeated a week later. We used pre-registered regions of interest in alERC and right middle temporal gyrus (rMTG), a candidate area for long term semantic memory. We hypothesized that Associative Coding would be stronger in Consistent than Inconsistent sequences and would shift from alERC to rMTG with time. We found that in Session 1, alERC showed Associative Coding in Consistent sequences, more so than Inconsistent ones, whereas rMTG showed no effects. In Session 2, this pattern flipped, and rMTG showed Associative coding in Consistent objects, more so than Inconsistent ones; alERC showed no effects. These findings demonstrate that both alERC and rMTG represent predictive relations and integrate information across learning experiences, but do so at different times, and suggest one way in which semantic memory sites may update with experience.

Disclosures: A. Leshinskaya: None. **M.A. Nguyen:** None. **C. Ranganath:** None.

Poster

P876. Entorhinal Cortex: Relational and Spatial Learning

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Program #/Poster #: P876.04

Topic: H.10. Human Learning and Cognition

Support: NIH grant R00MH103401

Title: Integrating region- and network-level contributions to episodic memory with multilevel structural equation modeling

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Abstract: An important challenge for cognitive neuroscience is understanding how individual brain regions function within the context of larger networks. In the context of episodic memory, activation in a posterior medial (PM) network has been consistently associated with successful recollection. Existing approaches have either considered how the network as a whole supports recollection or how the individual regions support recollection, but less is known about how

individual regions contribute to the network effect on memory. The goal of the present report was to bridge region- and network- focused approaches by using multilevel structural equation modeling (SEM) to simultaneously model the contributions of individual regions and the combined network to episodic remembering. Using data from a previously published fMRI study of 28 healthy young adults (Cooper & Ritchey, 2019), we first mapped trial-specific measures of regional activation onto a single latent variable representing network wide coactivation. We then mapped trial specific measures of memory for three distinct episodic features onto another latent variable, representing overall memory for each episode. Using these latent variables, we then simultaneously modeled the statistical dependency between each region and the overall memory latent variable, as well as the network latent variable and the overall memory latent variable. This approach allowed us to estimate a unique effect for each region in the network while also accounting for its membership within the larger network (Bolt et al. 2018). The results showed that the contributions of the PM network regions to memory were largely explained by the joint engagement of the network. However, two regions displayed extra-network contributions to memory, with the PHC positively predicting and the MPFC negatively predicting memory when controlling for their membership within the PM network. In a separate analysis, we additionally addressed whether the PM network may be composed of two related subnetworks, as has been recently suggested in the literature (Cooper et al., 2021; Barnett et al., 2021; Buckner & DiNicola, 2019). A bifactor model of PM subnetworks revealed that both the ventral and dorsal subnetworks supported high quality remembering, and the ventral did so uniquely when controlling for the contributions of the dorsal subnetwork, but not vice versa. Moreover, the bifactor model appeared to fit our data better than a single factor model. Overall, our approach provides for a more integrated understanding of the PM network, combining regional and network levels of analysis.

Disclosures: K. Kurkela: None. **R. Cooper:** None. **E. Ryu:** None. **M. Ritchey:** None.

Poster

P876. Entorhinal Cortex: Relational and Spatial Learning

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Program #/Poster #: P876.05

Topic: H.10. Human Learning and Cognition

Support: U19NS107609
K99MH120048494

Title: Parallel memory systems for efficient recognition

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Seattle, WA; [3]Dept. of Neurol., Univ. of California, Irvine, Irvine, CA; [4]Univ. of California Berkeley, Berkeley, CA

Abstract: Remembering the order of event occurrence is fundamental to episodic memory. Converging evidence suggests that at least two copies of events are encoded in parallel in distinct brain regions during memory formation. One system retains events with high fidelity and is hippocampal-dependent. The other system encodes the gist of events and has schematic information, monitored by the prefrontal cortex. How the two copies of memories are employed at recall is currently unknown. In this study, we investigated the neural dynamics within the hippocampus and the prefrontal cortex that support recognition of the temporal order of events from long-term memory. Epilepsy patients undergoing seizure monitoring with stereoencephalographic electrodes (sEEG; n=8) watched a movie and, after a 3-5 minutes delay, determined the order of movie frames that were presented in pairs. In half of the trials, the movie frames were from visually distinct scenes that occurred less than 11 seconds apart in the movie (i.e., close events); the remaining probes pairs occurred at longer intervals in the movie (i.e., far events). The far events were more likely to have distinct contexts, unlike the close events that shared a context, according to the annotation of event boundaries of the movie by an independent group of participants (n = 80). Recalling the order of close events had a longer response time (2 sec, SD = 0.79) than recalling far events (1.7 sec, SD = 0.73; paired-wise comparison $t(7) = 2.6$, $p = 0.03$). Using sEEG, we observed that theta power in the left anterior hippocampus was higher for correct recognition of the order of close events than for far events (peak at 6.5 Hz, FDR corrected $p<0.001$). In contrast, the beta power in the left medial orbitofrontal cortex (OFC) was higher for far compared to close events (peak at 22 Hz, FDR corrected $p = 0.019$). Previous studies have demonstrated impaired temporal order recognition in patients with damage to either the hippocampus or the OFC. The data presented here demonstrating the dynamics of the hippocampus and OFC activity at recognition support distinct contributions of these two regions at retrieval. Recalling memories with high temporal precision requires the hippocampus and longer intervals between events rely on the OFC and the overall movie schema to enhance recall. The benefits of multiple memory traces have been shown to contribute to long-term consolidation. We suggest that in the short-term multiple memory traces enhance the recognition of the order of events.

Disclosures: A. Jafarpour: None. **J. Lin:** None. **R.T. Knight:** None. **E.A. Buffalo:** None.

Poster

P876. Entorhinal Cortex: Relational and Spatial Learning

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NSF DGE-1745038

Title: Hippocampal and entorhinal contributions to naturalistic event context reinstatement

Authors: *A. I. DELARAZAN[1], A. B. KARAGOZ[1], M. E. MONTCHAL[2], M. A. YASSA[2], C. RANGANATH[3], Z. M. REAGH[1];
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Abstract: Remembering the temporal context of a past event is a defining feature of episodic memory. Medial temporal lobe (MTL) structures such the dentate gyrus and CA3 (DG/CA3) hippocampal subfield and the anterolateral entorhinal cortex (alERC) have previously been linked temporal memory precision. Hippocampal activity has furthermore been implicated in segmenting and representing naturalistic experiences as events. However, little is known about the way retrieved representations are shaped by the narrative structure of encoded events. In this study, participants viewed a 28-minute television episode and were later tasked with identifying, on a continuous timeline, when events occurred during high-resolution fMRI scanning. In line with prior results, we found increased activity in the hippocampus at transitions between events (event boundaries). Interestingly, this activation was significant at corrected thresholds only in the DG/CA3 hippocampal subfield. We next conducted representational similarity analyses to investigate whether activity patterns at retrieval were more similar for trials with images from the same encoding events versus different encoding events. Surprisingly, trials with images from the same encoding events were not more similar than the average inter-trial comparison. In contrast, pattern similarity throughout the MTL was greater for trials where temporal judgments subjectively grouped them into the same encoding event, regardless of the objective structure of the episode, suggesting construction of a subjective event context. We next examined encoding-retrieval similarity as a function of encoding event. Given that the precision of participants' temporal judgements determined event pattern reinstatement at retrieval, we incorporated a contrast of behavioral sensitivity into these analyses via a median split on test trials, resulting in retrieval patterns corresponding to precise and imprecise timeline judgments. DG/CA3 showed increased pattern similarity between encoding and retrieval for objective (but not subjective) event information for precise compared to imprecise trials. Conversely, alERC showed increased pattern similarity for subjective (but not objective) event context information for precise compared to imprecise trials. Finally, posteromedial entorhinal cortex (pmERC) showed a precise versus imprecise difference for both objective and subjective event contexts. These results provide evidence for representations of both objective and subjective event contexts in the MTL. Ongoing analyses investigate data-driven measures of event structure among different regions of interest.

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Poster

P876. Entorhinal Cortex: Relational and Spatial Learning

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ONR Grant N00014-14-1-0670 / N00014-16-1-2128
ONR Grant N00014-18-C-2067

Title: Correcting the hebbian mistake: toward a fully error-driven hippocampus

Authors: *Y. ZHENG, X. L. LIU, S. NISHIYAM, C. RANGANATH, R. C. O'REILLY; Ctr. for Neurosci., Univ. of California, Davis, Davis, CA

Abstract: The hippocampus plays a critical role in the rapid learning of new episodic memories. Many computational models propose that the hippocampus is an autoassociator that relies on Hebbian learning (i.e., “cells that fire together, wire together”). However, Hebbian learning is computationally suboptimal as it modifies weights unnecessarily beyond what is actually needed to achieve effective retrieval (“pattern completion”), causing more interference and resulting in a lower learning capacity. Our previous computational models have utilized a powerful, biologically plausible form of error-driven learning by contrasting local activity states at different points in time to create error signals in hippocampal CA1 (Ketz et al., 2013). New evidence regarding neurophysiological properties of the hippocampal subfield CA3 suggests that the hippocampus might be even more reliant on error-driven learning. Based on these findings, we propose a new model called Theremin (Total Hippocampal ERror MINimization) that incorporates biologically-based principles of error-driven learning in CA3 as well as CA1. In the model, CA3 pyramidal cells respond to the entorhinal cortex (EC) monosynaptic input (EC-CA3) prior to the EC disynaptic input through dentate gyrus (DG) (EC-DG-CA3). This signal delay gives rise to the temporal difference between the earlier EC-driven CA3 patterns and the later ones when DG inputs start to dominate, creating error signals to train the EC-CA3 connection and CA3-CA3 recurrent connection. In other words, DG serves as a teacher to CA3, correcting its patterns into more pattern-separated ones, thereby reducing interference. Results showed that Theremin, compared with our original model, has increasing model capacity and learning speed. Besides, we showed actication dynamics in hippocampal subregions over the course of a learning trial, and over multiple interations of learning. We will also present novel predictions from the model that can be tested in future studies.

Disclosures: Y. Zheng: None. **X.L. Liu:** None. **S. Nishiyam:** None. **C. Ranganath:** None. **R.C. O'Reilly:** A. Employment/Salary (full or part-time):; eCortex, Inc..

Poster

P065. Autism: Novel Cellular and Animal Models

Location: Virtual Only

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Program #/Poster #: P065.01

Topic: A.07. Developmental Disorders

Support: CONACYT CB-2015 255399
Postdoctoral Fellowship (CONACYT 24649)
Postdoctoral Fellowship (PRODEP 511-6/2019-15935)
UAM institutional fund

Title: C57 and CD-1 mice strains differ in behavioral and pharmacological responses after maternal immune activation as a preclinical model of schizophrenia/autism

Authors: *D. A. GONZÁLEZ-FRANCO[1], P. C. BELLO-MEDINA[2], M. PÉREZ-MORALES[1], J. GARCÍA-MENA[3], S. DIAZ-CINTRA[2], G. PACHECO-LÓPEZ[1];
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Abstract: Autism spectrum disorder and schizophrenia (ASD/SCZ) are neuropsychiatric diseases closely related to neurodevelopmental alterations. It has been shown that maternal immune activation (MIA) during gestation primes the central nervous system development inducing cognitive-emotional impairments in the offspring. One of the most popular and widely used approaches to study the effects on MIA is the maternal administration of the RNA viral mimic poly-inosinic/cytidylic acid [Poly(I:C)], which triggers the production and release of pro-inflammatory cytokines; IL-1β, IL-6, and TNF-α. Poly(I:C) treatment in MIA models results in numerous neurochemical and brain morphological abnormalities in the adult offspring. To date, most published preclinical research has been carried out in the mouse C57 inbred strain, therefore very little is known about the behavioral characteristics in other mice strains or outbreed mice models. In the present study, we administered poly(I:C) at gestational day 12 to induce MIA in pregnant C57 (inbred) and CD-1 (outbred) mice strains, followed by behavioral phenotyping as well as neuropharmacological sensitivity test of male and female offspring from both strains. Our results show an increase in the marble burying test in C57 males (repetitive and persevering behavior related to obsessive compulsive disorder), but unexpectedly, a decrease

was observed in CD-1 males in the same test. Data analyses also show decreased social interaction test, anhedonia, and sensitivity to the psychotomimetic drug amphetamine in males of both strains. Remarkably, MIA treatment did not induce any observable effect in the female offspring of both strains. Our results suggest that mouse strain and sex must be considered to analyze the effects of MIA in preclinical models of ASD/SCZ. However, despite the differences observed between the strains, it is possible to find consistent and robust effects of MIA treatment that allow identifying the symptoms of these disorders in the outbred strain for preclinical models. Our data also show that males of both C57 and CD1 strains are particularly more susceptible than females to display symptoms of these disorders after MIA, which agrees with epidemiological human evidence. Additional neurophysiological and neuromorphological studies are necessary for a better interpretation of the between-strains differences observed in this study.
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Poster

P065. Autism: Novel Cellular and Animal Models

Location: Virtual Only

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Program #/Poster #: P065.02

Topic: A.07. Developmental Disorders

Support: NIEHS Grant R00ES027869
deArce-Koch Memorial Endowment Fund

Title: Developmental Pyrethroid Exposure and Autism-related Behaviors in Prairie Voles

Authors: K. K. ZADE[1], J. A. CARLSON[1], B. P. KISTLER[2], C. NAWOR[2], M. A. CURTIS[1], F. NAGHAVI[1], K. L. NEIFER[1], *J. P. BURKETT[1];
[1]Dept. of Neurosciences, [2]Dept. of Med., Univ. of Toledo Col. of Med., Toledo, OH

Abstract: Autism Spectrum Disorder is a cluster of incurable neurodevelopmental disorders with a prevalence of 1 in 54 people. Although autism is commonly thought of as a genetic disorder, up to 50% of autism risk derives from environmental sources. Exposure to pyrethroid pesticides during pregnancy has recently been linked to autism risk by epidemiology studies. Our previous experiments in mouse suggest that developmental exposure to pyrethroid pesticides causes an autism-related phenotype, including decreased vocalizations, repetitive behaviors, learning deficits, and hyperactivity. In this study, we exposed female prairie voles to the EPA reference pyrethroid, deltamethrin, throughout pregnancy and lactation, and tested the resulting

offspring for autism-related phenotype. Route of administration mimicked human exposure by being consumed in food at a concentration below the EPA benchmark dose (3 mg/kg every 3 days in peanut butter). One pup per litter was used for each test in a behavioral battery that included ultrasonic vocalizations, marble burying, repetitive behaviors, 24-hour mobility, operant conditioning, partner preference, consoling behavior, and fear conditioning. Biological samples were collected for FOS immunochemistry and omics applications. Results will be discussed in the context of relevance to autism and other developmental disorders.

Disclosures: K.K. Zade: None. **J.A. Carlson:** None. **B.P. Kistler:** None. **C. Nawor:** None. **M.A. Curtis:** None. **F. Naghavi:** None. **K.L. Neifer:** None. **J.P. Burkett:** None.

Poster

P065. Autism: Novel Cellular and Animal Models

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Program #/Poster #: P065.03

Topic: A.07. Developmental Disorders

Title: An in vitro model to study the association of genetic diversity and neurological conditions reveals novel genes in Dyrk1a-related disorders and axonal injury linked to susceptibility and resilience

Authors: *D. CORTES[1], M. PERA[2];
[2]Res., [1]The Jackson Lab., Bar Harbor, ME

Abstract: Genetic differences play an important role in how individuals cope with the environment, making some individuals more resilient and others more susceptible to insults. Here we developed a model to study in a rapid and reliable fashion how genetic differences modify the outcome of neurological conditions. As a proof of concept for our model, we decided to study axonal injury/recovery and inhibition of Dyrk1a which is associated with autism, microcephaly, and mental disabilities. Our approach was to use the eight CC founder mouse-derived embryonic stem cells (mESCs) and differentiate them into neurons. Current protocols can only differentiate mESCs from the 129 strain or substrains but not others. For that reason, we developed a novel protocol to differentiate diverse strains with high efficiency and that works in humans stem cells as well. Minor modifications of the protocol create cerebral organoids or generate different neuronal subtypes. Upon neural differentiation, we found that DYRK1a inhibition differentially affects the eight CC founder mESC-derived neurons due to their genetic diversity. B6 strain lies on the susceptible end of the spectrum whereas WSB strain lies on the resilient end. Bulk RNA-seq analysis showed only 13 genes affected by the inhibition of Dyrk1a during neural precursor differentiation on WSB whereas more than 700 were affected on B6. A group of genes that has been independently associated with microcephaly, mental retardation, and Down syndrome was affected when Dyrk1a was inhibited. We also found that neurogenesis

and axonal growth differentially affected both strains. Regulatory network analysis showed Foxb1, Hoxa4, and Hoxd3 to be part of the transcriptional factors found in response to Dyrk1a inhibition and a possible cause for microcephaly. During maturation, cerebral organoids showed that DYRK1a inhibition affected gliogenesis in most strains and gene sets associated with the deposition of extracellular matrix. Electrical activity was more impaired on B6 compared to WSB which correlated with a downregulation of Scn5a on B6 compared to WSB. We conducted an in vitro axotomy model which showed that B6 could not grow axons back whereas WSB did. CCN family and hypoxia response genes were differentially expressed, and Ascl1, Olig2, and Creb5 were found to regulate the differential response. Overall, our model proved to be a reliable tool that confirmed previous results in other animal models and humans and discovered novel mechanisms and genes associated with Dyrk1a inhibition and axonal regeneration. This model can be used or adapted to other neurodevelopmental or neuronal disease-related questions.

Disclosures: D. Cortes: None. **M. Pera:** None.

Poster

P065. Autism: Novel Cellular and Animal Models

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Program #/Poster #: P065.04

Topic: A.07. Developmental Disorders

Support: NIEHS R00 ES027869
deArce-Koch Memorial Endowment Fund
University of Toledo - Masters in Biomedical Science - Bioinformatics Program

Title: Elucidating the effects of developmental pyrethroid pesticide exposure in mouse brain using a multiomics approach

Authors: *M. CURTIS, K. ALGANEM, A. IMAMI, K. L. NEIFER, R. SHUKLA, R. E. MCCULLUMSMITH, J. P. BURKETT;
Neurosciences, Univ. of Toledo Col. of Med. and Life Sci., Toledo, OH

Abstract: Autism Spectrum Disorder (ASD) is a cluster of incurable neurodevelopmental disorders with a prevalence of 1 in 54 people. Despite its high incidence and a rise in prevalence each year, less than 10% of autism cases have an identifiable cause. The recent CHARGE study has suggested that developmental exposure to pyrethroids, a common pesticide widely used due to its supposed safety, may increase autism risk. To evaluate the effects of developmental pyrethroid exposure, mouse dams were administered deltamethrin (or vehicle) during pregnancy and lactation. The offspring were raised to adulthood and euthanized for tissue collection. Split-sample transcriptomic, kinomic, and metabolomic analyses were performed on whole brain tissue, to provide a comprehensive understanding of the multi-modal biophenotype induced by

pyrethroid exposure. Results from the transcriptomics analysis revealed decreased expression of two genes, *per2* and *ciart*, which function to regulate circadian rhythm. Other transcriptional pathways were also significantly impacted by developmental exposure, including signal transduction pathways, interferon associated pathways, apoptosis, and cell cycle regulation. Results from kinomics analysis showed overactivity of casein kinase 1 (CK1), which plays a key role in circadian clock control by phosphorylating *per2*. Other kinases involved in neuronal development, axon specification, and neurotransmitter release were differentially active between the groups. The metabolomics results predicted a set of metabolites that were significantly different between the groups. Pathway analysis using these metabolites resulted in significantly enriched pathways for folate biosynthesis, tryptophan metabolism, xenobiotic metabolism by cytochrome P450s, and steroid hormone biosynthesis. The impacted pathways observed in our metabolomics results also significantly overlap with the metabolome signature of autism from human prefrontal cortex. Using a multiomics approach, we have elucidated and identified biological entities and pathways in the brain which are dysregulated by developmental pyrethroid exposure in the mouse. These findings have important implications for the ongoing widespread use of this pesticide in the human environment.

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Poster

P065. Autism: Novel Cellular and Animal Models

Location: Virtual Only

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Program #/Poster #: P065.05

Topic: A.07. Developmental Disorders

Title: Ghrelin Mitigates Acetaminophen (APAP)-Induced Developmental Abnormalities in Rats.

Authors: *J. GUSS DARWICH[1], J. A. HERRINGTON[1,2], *C. HARSHAW[3], A. M. BRIGANDE[1], E. B. LEIF[1], P. J. CURRIE[1];
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Abstract: Previous research suggests that early-life exposure to the analgesic acetaminophen (APAP) results in oxidative stress that increases the risk of developing autism spectrum disorder and attention-deficit hyperactivity disorder in adulthood. The neuropeptide ghrelin (GHR) has antioxidant properties and neuroprotectant therapeutic potential as shown in rodent models for Parkinson's Disease. We therefore tested whether GHR administration reduces behavioral abnormalities induced by early-life exposure to APAP. Seven-day old rat pups (N=36) each received subcutaneous injections of either APAP (51.97 mg/kg), GRH (1 mg/kg), GRH + APAP (at the aforementioned doses), or saline vehicle. In Open Field (OF) testing in adulthood, rats

that had been treated with APAP alone showed reliably less thigmotaxis (center:edge ratio as a measure of anxiety) than vehicle-treated rats. Rats treated with GRH in addition to APAP showed levels of thigmotaxis similar to vehicle-treated controls. Rats treated with APAP alone made significantly fewer rotations during OF testing than rats given both GRH + APAP. These findings suggest that (A) APAP-induced developmental changes can be induced following a single, early-life, allometrically-scaled dose, and (B) GHR may provide some neuroprotection against APAP-induced developmental damage.

Disclosures: J. Guss Darwich: None. **J.A. Herrington:** None. **C. Harshaw:** None. **A.M. Brigande:** None. **E.B. Leif:** None. **P.J. Currie:** None.

Poster

P065. Autism: Novel Cellular and Animal Models

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Topic: A.07. Developmental Disorders

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McDonnell International Scholars Academy
The Jakob Gene Fund

Title: A novel mouse model of autism spectrum disorder (ASD)-related MYT1L Syndrome displays autistic-like behaviors and altered brain development

Authors: *J. CHEN[1], X. GE[2], K. B. MCCULLOUGH[1], K. K. NOGUCHI[3], J. R. GARBOW[2], J. N. CONSTANTINO[3], H. W. GABEL[4], S. E. MALONEY[3], J. D. DOUGHERTY[1];
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Abstract: Human studies have identified heterozygous mutations on MYT1L, a transcriptional factor, as the cause for ASD-related MYT1L Syndrome. However, MYT1L's role in brain development and function remains unknown. Here, we established the first mouse model of germline MYT1L mutation to investigate its loss of function *in vivo*. First, we showed that $MYT1L^{+/-}$ mice exhibited various phenotypes reminiscent to those found in MYT1L Syndrome patients, including hyperactivity, social deficits, obesity, and microcephaly. Next, by performing ATAC-seq and RNA-seq at different developmental time points, we found MYT1L loss results in genomic structure and transcriptional changes across pathways for neurogenesis and neuronal maturation. At last, we validated decreased neuronal proliferation during mouse embryonic brain development upon MYT1L loss. Those findings suggest MYT1L loss impairs brain development

and function, providing molecular and cellular mechanisms underlying the MYT1L Syndrome pathogenesis.

Disclosures: J. Chen: None. **X. Ge:** None. **K.B. McCullough:** None. **K.K. Noguchi:** None. **J.R. Garbow:** None. **J.N. Constantino:** None. **H.W. Gabel:** None. **S.E. Maloney:** None. **J.D. Dougherty:** None.

Poster

P065. Autism: Novel Cellular and Animal Models

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P065.07

Topic: A.07. Developmental Disorders

Support: NSERC

Title: The Effect of Propionic Acid on Habituation to Social Odours by Adult Male Rats

Authors: *C.-D. TYSON, *M. KAVALIERS, K.-P. OSSENKOPP;
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Abstract: Accumulating evidence suggests that disturbances of the gastrointestinal tract involving metabolites of the gut microbiome play a role in the etiology of autism spectrum disorder (ASD). Specifically, the short-chain fatty acid, propionic acid (PPA), is of interest as it can cross both the gut-blood and blood-brain barriers, and induce central effects. One way ASD may contribute to social deficits, is through impaired habituation to sensory and social stimuli. To date, there has been no consideration of the effects of PPA on the habituation to sensory social cues. The present study examined effects of systemic administration of PPA in male adult Long Evans rats, and their ability to habituate to and discriminate between unfamiliar social and non-social odour cues. To ensure sufficient PPA was in the central nervous system to promote behavioural effects, prior to test days, rats were given intraperitoneal (i.p.) injections of 500 mg/kg PPA or phosphate buffered saline (PBS) for 4 successive days. On each of the baseline and 4 test days, rats received 500 mg/kg i.p. injections of PPA or PBS. On the baseline day, rats were exposed to clean bedding. To examine habituation, the next 3 test days, rats were either exposed to a repeated social odour cue from one stranger rat's bedding, or a non-social odour cue of diluted vanilla extract. On the last test day, a novel odour from a new stranger rat's bedding was introduced for social groups, and diluted almond extract was introduced for non-social groups. Behaviour was video recorded for 10 minutes while rats were in a 2-chambered open-field, with the odour in a petri dish on one chamber, and the other chamber empty. The open-field apparatus detected all vertical and horizontal activity via infrared beams, and total self-grooming, approach toward the odour cue and sniffing of the odour cue were manually scored from videotapes. Across baseline and test days, PPA treated rats showed significantly more self-

grooming than PBS rats in both social and non-social groups. Across the first 3 test days, the PPA-social group showed trends of reduced habituation compared to the PPA non-social group concerning duration near the odour cue, number of entries into the odour exposure area, and total horizontal activity. Total rest time was also significantly higher for PPA groups compared PBS groups during baseline and habituation days. PBS rats also showed a higher increase in sniffing than PPA rats when the novel odour was introduced on the last test day, compared to the previous day with the familiar odour. These results suggest that one means whereby PPA may contribute to social deficits in ASD is by impairing habituation to sensory social cues.

Disclosures: C. Tyson: None. **M. Kavaliers:** None. **K. Ossenkopp:** None.

Poster

P065. Autism: Novel Cellular and Animal Models

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Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P065.08

Topic: A.07. Developmental Disorders

Support: NIH Grant HRD-1826490
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Title: Characterizing autism-like social behaviors in Drosophila melanogaster.

Authors: *E. ALMS[1], D. LENT[2];
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Abstract: Studies have shown individuals diagnosed with autism can present with impairments and changes in socialization behaviors. These behavioral differences can include, among other things, increased aggression, impaired communication, and, interestingly in some individuals, better socialization between autistic peers. Autism, like many other neurological conditions, is diagnosed most often using behavioral surveys rather than medical confirmation testing. However, Fragile X Syndrome (FXS) is the most prevalent monogenic cause of hereditary autism currently known and it is one of the most commonly studied biological underpinnings in the D. melanogaster models of autism. In humans, FXS is the result of a mutation of the fragile X mental retardation 1 (FMR1) gene which has homolog in the D. melanogaster (dFMR1). The purpose of this study was to characterize the effect of selective dFMR1 knockdown in different neural circuitry on courtship social behaviors. Specifically, we looked at the effects of discriminatory mating patterns between flies with and without dFMR1 knockdown and within flies with or without dFMR1 knockdown. There were significant differences in the socialization behaviors of dFMR1/wildtype pairings compared to dFMR1/dFMR1 and wildtype/wildtype pairings. The observed differences in socialization in our fruit fly model have helped us better understand the autism-like socialization impairments and the improved within-group

socialization that has been observed in some individuals with autism. The latter is of particular interest as it has been suggested that the autistic individuals that socialize better with their autistic peers rather than with neurotypicals is related to a phenomenon called the double empathy problem. If the results are supported this may provide evidence that in D. melanogaster, there may be a neurobiological foundation for this phenomenon.

Disclosures: E. Alms: None. **D. Lent:** None.

Poster

P065. Autism: Novel Cellular and Animal Models

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P065.09

Topic: A.07. Developmental Disorders

Title: Impairments in behavioral flexibility in the BTBR mouse model of autism spectrum disorder.

Authors: *B. ALVAREZ, C. MORALES, C. CAVAZOS, L. AMODEO, D. AMODEO; Psychology, California State University, San Bernardino, San Bernardino, CA

Abstract: Individuals diagnosed with autism spectrum disorder (ASD) display impairments in behavioral flexibility, as evidenced by deficits in probabilistic reversal learning. Similarly, the BTBR T+Itpr3tf/J (BTBR) mouse model of ASD shows a similar deficit in probabilistic reversal learning, when compared to C57BL/6J control mice when utilizing a spatial maze learning paradigm. In addition, many of these studies have failed to include female subjects. The first goal of these experiments was to examine the reproducibility of these findings using mouse operant chambers. We predicted that the impairments found with the spatial T-maze would parallel results using operant chambers. Secondly, we hypothesized that female BTBR mice would express similar impairments to male BTBR mice. Mice were tested in operant discrimination task using an 80/20 probabilistic reinforcement procedure. Mice were tested on a two-choice operant task, each mouse was tested on acquisition and then reversal learning across several days. Mice learned to obtain a sucrose pellet reinforcement from the magazine once a “correct” nose poke was made (reinforced on 80% of trials) compared with the “incorrect” nose poke (reinforced on 20% of trials). The learning criterion in both phases was choosing the ‘correct’ location on 80% of trials. For the initial operant discrimination, female mice regardless of strain required less days to reach learning criterion than male mice. For both days needed to reach criterion and total trials needed to reach criterion, both female and male BTBR mice were impaired. This impairment parallels findings utilizing a spatial learning task. Further error analysis shows that the impaired behavioral flexibility was due to an inability to maintain a new choice pattern once the reversal was made, rather than an initial inability to inhibit the initial learned choice behavior. These findings demonstrate that the behavioral inflexibility found in

BTBR mice is persistent and resistant to changes in behavioral paradigms. Together these findings further support the testing of this strain in the hopes of discovering of new treatments aimed a reducing behavioral inflexibility.

Disclosures: B. Alvarez: None. **C. Morales:** None. **C. Cavazos:** None. **L. Amodeo:** None. **D. Amodeo:** None.

Poster

P065. Autism: Novel Cellular and Animal Models

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Program #/Poster #: P065.10

Topic: A.07. Developmental Disorders

Support: R35 NS111573
R01 MH104450
SFARI Program of the Simons Foundation

Title: Autism-associated mutations in kv7 channels induce gating pore current

Authors: *T. M. GAMAL EL-DIN[1], T. LANTIN[2], C. TSCHUMI[2], B. JUAREZ[2], M. QUINIAN[2], J. LI[2], L. S. ZWEIFEL[1], W. A. CATTERALL[2];
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Abstract: Autism spectrum disorder (ASD) adversely impacts >1% of children in the U. S., causing social interaction deficits, repetitive behaviors, and communication disorders. Genetic analysis of ASD has advanced dramatically through genome sequencing, which has identified >500 genes with mutations in ASD. The voltage-gated potassium (K_V) channel K_V7 (KCNQ) ranked high among the ion channel genes having mutations in individuals with neurodevelopmental disorders. The K_V7 channel in brain is typically a heterotetramer of $K_V7.2$ and $K_V7.3$ subunits. These channels are tonically active at the resting membrane potential to regulate voltage sensitivity, frequency, and pattern of action potential firing in brain neurons. Like other K_V channels, K_V7 is composed of four subunits having six transmembrane segments (S1-S6). The S1-S4 segments form the voltage sensor, whereas the S5 and S6 segments form the pore. Mutations that alter arginine gating charges in the voltage sensor of the voltage-gated potassium channel K_V7 (KCNQ) are among those frequently associated with ASD. We hypothesized that these gating charge mutations would induce gating pore current (also termed ω-current) by causing an ionic leak through the mutant voltage sensor. Unexpectedly, we found that wildtype K_V7 conducts outward gating pore current through its native voltage sensor at positive membrane potentials, owing to a glutamine in the third gating charge position. In bacterial and human K_V7 channels, gating charge mutations at the R1 and R2 positions cause inward gating pore current through the resting voltage sensor at negative membrane potentials,

whereas mutation at R4 causes outward gating pore current through the activated voltage sensor at positive potentials. Remarkably, CRISPR/Cas expression of the K_V7/R2C ASD-associated mutation *in vivo* in dopamine neurons of mice disrupts action potential generation and repetitive firing. Overall, our results reveal native and mutant gating pore current in K_V7 channels and implicate altered control of action potential generation by gating pore current through mutant K_V7 channels as a potential pathogenic mechanism in autism.

Disclosures: T.M. Gamal El-Din: None. **T. Lantin:** None. **C. Tschumi:** None. **B. Juarez:** None. **M. Quinian:** None. **J. Li:** None. **L.S. Zweifel:** None. **W.A. Catterall:** None.

Poster

P166. Oligodendrocytes: Development and Aging

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P166.01

Topic: B.11. Glial Mechanisms

Support: 1R01NS109227

Title: The DYT6 dystonia transcription factor THAP1 regulates myelination and ECM homeostasis

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Abstract: The development and function of the nervous system is profoundly influenced by interactions of neurons with glial cells and the extracellular matrix (ECM). Mechanisms regulating the maturation of oligodendroglial progenitor cells (OPCs) into myelinating cells to facilitate CNS myelination play an essential role in circuit plasticity, including motor learning. Prior studies have established that OPC maturation into myelinating cells is acutely sensitive to ECM content and composition. Yet we understand little about the cellular mechanisms regulating the synthesis and composition of the brain ECM. Our work has identified that THAP1, a transcription factor whose loss of function leads to the neurodevelopmental movement disorder DYT6 dystonia, drives OPC development by regulating the breakdown of glycosaminoglycans (GAGs), which are major ECM components. Conditional deletion of *Thap1* from the oligodendrocyte (OL) lineage significantly impairs CNS myelination by restraining the maturation of OPCs to myelin producing cells. Our studies show that THAP1 modulates the ECM content produced by the OPCs by binding to and regulating the *Gusb* gene, which encodes for β-glucuronidase, a GAG-catabolic lysosomal enzyme. Our studies of the transcription factor THAP1 in the OL lineage have identified that GAGs produced and secreted by OPCs act in an

autocrine fashion to inhibit their lineage progression, highlighting a unique mechanism regulating the pool of mature oligodendrocytes.

Disclosures: D. Yellajoshyula: None. **A.E. Rogers:** None. **S.S. Pappas:** None. **W.T. Dauer:** None.

Poster

P166. Oligodendrocytes: Development and Aging

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Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P166.02

Topic: B.11. Glial Mechanisms

Support: DIRP, NIMH, NIH
DIRP, NICHD, NIH

Title: Oligodendrocyte-mediated myelin plasticity

Authors: *S. PAJEVIC[1], D. PLENZ[1], P. J. BASSER[2], R. D. FIELDS[2];
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Abstract: The most efficient mechanism for adjusting the timing of signals among different neurons in vertebrate brains is by altering conduction velocity through the dynamic and adaptive remodeling of myelin, i.e., myelin plasticity (MP). Oligodendrocytes (OL) play an important role in MP, but the precise feedback mechanisms for adjusting the degree of axonal myelination and timing are currently not known. The models that we propose here address this problem and are motivated by the fact that a single OL cell preferentially extends its processes to multiple axons rather than myelinating the same axon multiple times. We propose two mathematical but biologically plausible models of oligodendrocyte-mediated myelin plasticity (OMMP), in which we explore the role OLs can play in mediating synchronization of signals traveling via different axonal pathways they myelinate: 1) a *passive model* (PM), in which the relative timings of axonal spikes at different processes of oligodendrocytes are driving/influencing myelination; and 2) an *active model* (AM) in which the recently observed ability of some OL cells to produce their own spikes is used as a reference signal for synchronization. The essential features of these models are relative timings and temporal profiles of the release of various “factors” within the OL cells in response to axonal spikes. The passive model allows oligodendrocytes to effectively mediate synchronization of signals, while requiring only two factors, one that promotes the myelination (local, having different concentration on each of the OL processes), and another that catalyzes the release of the myelinating factor (global/somatic, having the same concentration for a given OL cell). Assuming that the demyelination rate is constant, in its simplest form the PM has only $3+n_a$ dynamical variables per OL cell, where n_a is the number of axons the OL myelinates, whereas the AM model required $6 + 2\ n_a$ variables to have stable dynamics. The full

model consists of a sequential "chain" of n_o OL cells in which the myelination pattern across all OLs is prescribed by the myelination matrix (n_o x n_a). In this work we present results of simulations in which we test the ability of OL chains to recover from structural perturbations in conduction delays and re-synchronize the signals with consistent timing differences. The initial spread of arrival times (varied between 5 and 20 ms) can be reduced to only a few milliseconds (1-4 ms), yielding better results for low spiking rates (<20 Hz). We also show that both OMMP models leave the arrival time spread unchanged when independent and random (Poisson) spike trains are transmitted through the chain.

Disclosures: S. Pajevic: None. **D. Plenz:** None. **P.J. Basser:** None. **R.D. Fields:** None.

Poster

P166. Oligodendrocytes: Development and Aging

Location: Virtual Only

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Program #/Poster #: P166.03

Topic: B.11. Glial Mechanisms

Support: SyNergy Excellence Cluster EXC2145, Projekt ID390857198, and RO 4076/3-2)

Title: White matter aging drives microglial diversity

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Abstract: Aging results in gray and white matter degeneration, but the specific microglial responses are unknown. Using single-cell RNA sequencing from white and gray matter separately, we identified white matter-associated microglia (WAMs), which share parts of the disease-associated microglia (DAM) gene signature and are characterized by activation of genes implicated in phagocytic activity and lipid metabolism. WAMs depend on triggering receptor expressed on myeloid cells 2 (TREM2) signaling and are aging dependent. In the aged brain, WAMs form independent of apolipoprotein E (APOE), in contrast to mouse models of Alzheimer's disease, in which microglia with theWAMgene signature are generated prematurely and in an APOE-dependent pathway similar to DAMs. Within the white matter, microglia frequently cluster in nodules, where they are engaged in clearing degenerated myelin. Thus, WAMs may represent a potentially protective response required to clear degenerated myelin accumulating during white matter aging and disease.

Disclosures: S. Besson-Girard: None. **T. Kaya:** None. **S. Safaiyan:** None. **L. Liu:** None. **M. Simons:** None. **H. Ji:** None. **O. Gokce:** None.

Poster

P166. Oligodendrocytes: Development and Aging

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P166.04

Topic: B.11. Glial Mechanisms

Title: Role of Tim-2 in central nervous system myelination

Authors: *Q. W. WADE, E. B. NEELY, J. R. CONNOR;
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Abstract: Myelin, which is formed by oligodendrocytes (OLs) in the central nervous system (CNS), is crucial for efficient signal transduction and neuronal function. Iron is a critical micronutrient for OLs due to its role as a cofactor for myelin synthesis, and it is well-known that OLs are the highest iron staining cells in the brain. Inadequate iron delivery to OLs frequently results in severe and long-lasting neurological deficits that are attributed to hypomyelination. Our laboratory and others have identified H-ferritin (Fth), traditionally considered solely an iron storage protein, as the primary iron delivery protein to OLs. We have also found that H-ferritin utilizes a novel receptor on rodent OLs, T-cell immunoglobulin and mucin domain-containing protein-2 (Tim-2). To determine the importance of H-ferritin-mediated iron delivery through Tim-2 on OLs for myelination, we have generated *Timd2$^{fl/fl}$ Plp1-Cre/ERT* conditional knockout mice to eliminate Tim-2 expression specifically from OLs following consecutive tamoxifen injections. We hypothesize that removal of Tim-2 from OLs will lead to decreased H-ferritin uptake, which will in turn lead to insufficient myelination. In this study, we observed that *Timd2$^{fl/fl}$ Plp1-Cre/ERT* mice injected with 75 mg/kg tamoxifen on post-natal days 7-9 to induce the knockout displayed a trend towards poorer motor performance on the rotarod as measured by increased number of falls and decreased latency to fall. Moreover, there was a statistically significant reduction in myelin basic protein (MBP) and proteolipid protein (PLP) in addition to a decrease in myelin staining in the corpus callosum of Tim-2 knockout animals compared to controls. Essentially, the data from this study indicates that myelin is negatively affected following Tim-2 knockout from OLs during development.

Disclosures: Q.W. Wade: None. **E.B. Neely:** None. **J.R. Connor:** None.

Poster

P166. Oligodendrocytes: Development and Aging

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Topic: B.11. Glial Mechanisms

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Title: Oligodendrocyte precursor cells prune axons in the mouse neocortex

Authors: *J. BUCHANAN[1], L. ELABBADY[1], F. C. COLLMAN[2], N. L. JORSTAD[3], T. E. BAKKEN[3], C. OTT[4], J. C. GLATZER[5], A. L. BODOR[6], D. BRITTAIN[2], D. J. BUMBARGER[6], G. MAHALINGAM[1], S. SESHAMANI[9], C. M. SCHNEIDER-MIZELL[6], M. M. TAKENO[6], R. TORRES[1], W. YIN[2], R. D. HODGE[7], S. DORKENWALD[10], D. IH[10], N. KEMNITZ[10], K. LEE[10], R. LU[10], T. MACRINA[10], N. L. TURNER[10], J. WU[10], E. LEIN[3], H. SEUNG[10], D. E. BERGLES[11], R. REID[1], N. M. DA COSTA[8];
[1]Neural Coding, [3]Human Cell Types, [2]Allen Inst. for Brain Sci., Seattle, WA; [4]Janelia Res. Campus, Ashburn, VA; [5]Cell. and Mol. Med. Grad. Program, Johns Hopkins Med., Baltimore, MD; [6]Neural Coding, [7]Cell Types Program, [8]Allen Inst. For Brain Sci., Seattle, WA; [9]Synapse Biol., Allen Inst., Seattle, WA; [10]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ; [11]Johns Hopkins Univ. Sch. Med., Baltimore, MD

Abstract: Oligodendrocyte precursor cells (OPCs) play a crucial role in the CNS by serving as progenitors for oligodendrocytes, which are mobilized to ensure proper developmental myelination, adaptive myelination in response to learning, and myelin repair following injury and disease. However, the early emergence of these OPCs during development, their highly dynamic nature and their persistence within gray matter throughout life has raised the possibility that these cells perform other functions in neural circuits. Assessing their possible non-progenitor roles has been difficult, as there is limited information about the structure of OPCs in the intact brain.

To help define the function of OPCs in the developing CNS, we used high throughput serial section EM and computational segmentation to examine the detailed ultrastructure of OPCs in mouse visual cortex (aged P36 and P49), providing the first complete 3D reconstructions of these cells. Unexpectedly, we discovered that the fine processes of OPCs frequently surrounded terminal axon branches and included numerous lysosomes and phagolysosomes (PLs) containing fragments of axons and presynaptic terminals, containing 40 nm vesicles within their chambers. Single- nucleus RNA sequencing and DNA-methylation analyses indicated that cortical OPCs express key phagocytic genes, as well as methylated neuronal transcripts, consistent with active axonal engulfment.

To compare their phagocytic activity to microglia, long considered the primary phagocytes in the brain, we counted PLs in 18 microglial cells from both datasets and compared the numbers and density of PLs to those in 18 OPCs. This analysis showed PLs were ~10x more abundant in OPCs at these ages than other glia. Immunostaining using anti-LAMP-2 antibody revealed that OPCs acutely isolated from developing visual cortex contained numerous lysosomes, further indicating that these cells are highly phagocytic at this age. PLs in OPCs declined with age and lineage progression, suggesting that OPCs contribute to the refinement of neuronal circuits during later phases of cortical development by pruning synapses and terminal axonal branches.

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Poster

P166. Oligodendrocytes: Development and Aging

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Program #/Poster #: P166.06

Topic: B.11. Glial Mechanisms

Support: NIH Grant R25 NS 80686

Title: The Effect of Jedi-1, an engulfment receptor on Oligodendrocyte Lineage cell Development and Proliferation

Authors: *J. TEPAN[1], B. D. CARTER[2], V. MORRISON[3];
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Abstract: Jedi-1 is an engulfment receptor that aids in phagocytosis and clearance of apoptotic debris in the peripheral nervous system. In the central nervous system of Jedi-1 knockout (KO) mice, phagocytic clearance is disrupted and proliferation decreases in the subventricular zone (SVZ), a neurogenic region known for high levels of proliferation and apoptosis Here, we ask whether Jedi-1, through its effects on the SVZ, influences oligodendrocyte lineage cell (OLC) development. OLCs arise from the SVZ and colonize the corpus callosum, where they ensheath

and insulate axons, thereby increasing action potential velocity. We previously found that despite a decreased number of neural precursors in the KO SVZ, more became OLCs than in wild types (WT) at postnatal day 7 (P7). Thus, we hypothesized that adult KO mice would have a larger pool of OLCs relative to WT. Immunofluorescent labeling of the oligodendrocyte (OL) lineage marker, OLIG2+ in brain sections showed no significant difference between genotypes in total OLCs in the corpus callosum at P28. Given this finding, the increase in OLIG2+ cells at P7 could represent a transient compensatory increase in oligodendrogenesis, perhaps to compensate for reduced neural precursors. There might also be increased proliferation within the OLC population that could explain comparable numbers of OLC in the adults. To address this, we stained for EdU, a fluorescent nucleotide analog incorporated into cells dividing at the time of EdU administration,and quantified the proportion of proliferating OLCs. Increasing OL production postnatally can provide insight on treating demyelinating diseases such as multiple sclerosis.

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Poster

P166. Oligodendrocytes: Development and Aging

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Topic: B.11. Glial Mechanisms

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Title: Aging-dependent transcriptomic changes in oligodendrocyte precursor cells

Authors: *D. HEO[1], V. N. DOZE[3], L. A. GOFF[1,2,4], D. E. BERGLES[1,4];
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Abstract: OPCs represent an abundant, highly dynamic, widely distributed population of glial cells in the CNS that are responsible for generating myelinating oligodendrocytes during development. These cells are also mobilized to form new myelin in the adult CNS in response to environmental and behavioral changes, and play a crucial role in regenerating myelin in multiple sclerosis (MS). However, the rates of OPC proliferation and differentiation decline dramatically with aging, impairing homeostasis, myelin repair, and potentially adaptive myelination during learning. The mechanisms that cause this shift in behavior are not yet known. In Alzheimer's disease (AD), there is evidence that OPCs can become senescent, characterized by increased

immunoreactivity of p21 and p16, key proteins involved in inhibiting cyclin-dependent kinases during cellular division. In MS, similar patterns of OPC and OL senescence have been identified, suggesting that aging-related changes in OPCs may contribute to these neurodegenerative diseases. To determine how OPCs change with age, we isolated these cells using FACS from the cerebral cortex of a novel transgenic mouse line that expresses EGFP under the endogenous promoter of matrilin-4 (*Matn4*-mEGFP), allowing us to enrich for these cells with high purity. We then performed single-cell RNA sequencing from more than 30,000 OPCs from mice aged P30, P180, P360, and P720. Gene expression clustering analysis revealed three major subtypes of cortical OPCs: cycling, quiescent, and differentiating. Cycling OPCs were further subclassified into three different populations based on their position in the cell cycle, the proportion of which declined with aging. We then performed aging-dependent differential gene expression analysis on different subsets of OPCs (cycling, quiescent and differentiating) and identified co-regulated gene patterns. Unexpectedly, quiescent OPCs were transcriptionally stable over the course of aging, suggesting that these cells have an intrinsic potential to self-renew and rarely become senescent in the naïve brain. We then performed single-cell ATAC-seq to determine aging-dependent changes in chromatin accessibility that may ultimately contribute to the decreased potential for differentiation and proliferation with aging. These datasets provide a valuable reference of OPC gene expression patterns to help define their behavior in various physiological and pathological contexts. Further, they will provide a deeper understanding of the homeostatic roles performed by these cells, and reveal new approaches for restoring their regenerative potential in aging-associated diseases and injury.

Disclosures: D. Heo: None. **V.N. Doze:** None. **L.A. Goff:** None. **D.E. Bergles:** None.

Poster

P166. Oligodendrocytes: Development and Aging

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P166.08

Topic: B.11. Glial Mechanisms

Support: NSERC CGS-M
NSERC RGPIN-2020-06403

Title: Sex differences in visual cortex development: is there a role for myelin?

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Abstract: There are well-known sex differences in visual perception, and the visual cortex (V1C) is >20% larger in males than females. Nevertheless, the issue of sex differences in V1C development has not been well-studied. For example, sex hormones such as estradiol enhance

oligodendrogenesis which promotes myelin production. Also, various disorders that affect cortical myelin such as multiple sclerosis, and bipolar disorder are more prevalent in females than males, suggesting sex-specific disparities. Myelin is a complex multiprotein that has been shown to serve more than one role in V1C development and plasticity. In the critical period, an increase in myelin-derived signaling puts the 'brakes' on developmental plasticity. Paradoxically, in the adult V1C, myelin increases in response to enriched sensory experience suggesting that it facilitates plasticity. Together, these raise the question of whether the molecular composition of myelin differs by age and sex? To address this, we analyzed the development of a network of myelin-associated genes (n=58, e.g., MBP, PLP1, MAG, MOG) using a developmental transcriptomic database from post-mortem tissue samples of the human primary V1C (n=48, samples, F=21, M=27, age range: 4 months -80 years). First, we characterized the lifespan changes for the network of genes by fitting locally estimated scatterplot smoothing (LOESS) curves with 95% confidence intervals for each gene and sex pair (n=116). We noticed that some genes showed monotonic increases or decreases across the lifespan. In contrast, others had an undulating pattern unique to adolescence coinciding with the end of the sensitive period in the human V1C. Second, we conducted unsupervised hierarchical clustering of the myelin gene development and found 8 distinct trajectories that describe the developmental profile of myelin in human V1C. We listed the genes in each trajectory cluster and found minimal overlap between the sexes. For example, the major components of myelin, MBP and PLP1, had different developmental trajectories for females and males. This trajectory analysis identified robust age- and sex-related differences in the development of myelin genes in human V1C. These novel findings suggest that myelin may play different roles in transitioning from juvenile to adult plasticity in V1C of females and males.

Disclosures: L. Monteiro: None. **D. Singh:** None. **K.M. Murphy:** None.

Poster

P166. Oligodendrocytes: Development and Aging

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Program #/Poster #: P166.09

Topic: B.11. Glial Mechanisms

Support: NINDS grant R01NS104021
NYSTEM Stem Cells in Regenerative Medicine Fellowship #C302090G

Title: Oscillatory store-operated calcium signals regulate human oligodendrocyte progenitor stem cell fate

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Abstract: In multiple sclerosis (MS), destruction of myelin sheaths and insufficient repair contributes to axonal degeneration and neurological dysfunction. Endogenous remyelination requires the differentiation of adult oligodendrocyte progenitor cells (OPCs). As such, pharmacologic interventions for MS requires an understanding of molecular regulators of human OPC (hOPC) maturation. We have previously shown that the $G\alpha_q$ muscarinic M_3 receptor (M_3R) inhibits hOPC differentiation, and conditional deletion of M_3R in OPCs promotes remyelination. We hypothesized that Ca^{2+} signals downstream $G\alpha_q$-coupled-receptor (G_qR) signaling regulates hOPC cell fate. We exposed hOPCs to a panel of selective ligands that target G_qRs identified by RNA-seq. Only muscarinic agonist oxotremorine (Oxo-M) and the metabotropic glutamate receptor 5 ($mGluR_5$) agonist CHPG induced prolonged oscillatory $[Ca^{2+}]_i$ in hOPCs and blocked hOPC differentiation while another G_qR agonist had no effect. Oscillatory $G\alpha_q$-$[Ca^{2+}]_i$ was also dependent on store operated calcium entry (SOCE) and was reduced following treatment with SOCE antagonists and siRNA-mediated knockdown (KD) of either STIM1 or STIM2. Interestingly, STIM2 KD reduced $[Ca^{2+}]_i$ oscillatory frequency and abolished effects of muscarinic agonist on differentiation. To further examine the role of oscillatory SOCE, we used optogenetic STIM lentivirus to directly induce SOCE by blue light stimulation at various frequencies. Following long-term live cell imaging, we observed a frequency-dependent effect of SOCE activation resulting in blockade of hOPC differentiation and stimulation of proliferation. Lastly, in our preliminary *in vivo* studies using conditional deletion of SOCE components *Stim1/2* in $NG2^+$ OPCs, we found *Stim1/2* ablation increases oligodendrocyte (OL) generation following demyelination. These findings afford insights into mechanisms that regulate hOPC proliferation and differentiation, suggesting a key role for SOCE in the regulation of OL cell fate.

Disclosures: R.A. Seidman: None. **J.J. Polanco:** None. **J.E. Broome:** None. **F.J. Sim:** None.

Poster

P166. Oligodendrocytes: Development and Aging

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Program #/Poster #: P166.10

Topic: B.11. Glial Mechanisms

Title: Role of PARG in oligodendrocyte differentiation during CNS development

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Abstract: Oligodendrocyte differentiation and myelination are essential for the normal function of the central nervous system (CNS). Defects of these two events result in saltatory nerve conduction disruption, axonal degeneration and neurological disabilities such as periventricular leukomalacia and multiple sclerosis. Hence, understanding the mechanisms and identification of critical regulators underlying OL development and myelination facilitates the development of therapeutic targets for CNS demyelinating diseases. We have previously reported that Poly(ADP-ribose) polymerase 1 (PARP1) plays an essential role in oligodendroglial lineage progression through its enzymatic activity mediated PARylation, a process of covalently attaching poly (ADP-ribose) unites to target proteins. However, the rapid PAR turnover is catalyzed by the PAR degrading enzyme Poly(ADP-ribose) glycohydrolase (PARG). Our *in vivo* study showed that stabilizing PARylation by PARG inhibitor (PDD 00017273) treatment augmented PARylation through dampening PAR degradation. The number and differentiation of oligodendrocyte were significantly increased in the corpus callosum of PDD treated mice compared with that of control mice. For *in vitro* study, both PDD treatment and siRNA-mediated PARG knockdown in primary cultured rat OPCs significantly increased the expression of myelin genes and pro-differentiation factor suggesting that PARG regulates OPC differentiation in a cell-autonomous manner. Therefore, our studies demonstrate that inhibiting PARG activity or decreasing PARG expression is sufficient to promote OPC differentiation and suggest that PARG-mediated pathway may be a potential therapeutic target for myelin repair in demyelinating disorders.

Disclosures: Y. Wang: None. **S. Zhang:** None. **B. Kim:** None. **F. Guo:** None.

Poster

P213. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: Virtual Only

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Program #/Poster #: P213.01

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

Support: Regis College Kaneb Grant

Title: White matter integrity of Alzheimer's disease characterized by diffusion tensor imaging

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Abstract: The white matter degeneration is suggested to predict the early sign of Alzheimer disease (AD). Changes in AD are reflected on the microstructure of white matter tract, for example the degree of myelination. The purpose of this research is to study the changes of white matter tract integrity, the specific Diffusion Tensor Imaging (DTI) measures, in AD patients in

comparison to control brains.We have studied 4 DTI measures, Mean Diffusivity (MD), Fractional Anisotrophy (FA), Radial Diffusivity (RD) and Axial Diffusivity (AxD) in 42 AD patients and 42 controls in 12 main brain white fiber tract regions on both hemispheres, corpus callosum, fornix, cingulum hippocampus, uncinate, internal and external capsule, corona radiata, cerebellar peduncle, longitudinal fasciculus, fronto-occipital fasciculus, striatum, thalamus radiation and corticospinal tract. The following brain regions show a bilateral significant increase in MD, RD, AxD and decreased FA among AD patients: fornix, corpus callosum, anterior corona radiata and cingulum hippocampus. Adjusted for other covariates, education is not associated with DTI measurers ($p > 0.05$). Females had lower MD values, in certain brain regions such as cerebral peduncle, fornix and superior longitudinal fasciculus. Elder people had higher MD values in most DTI measures. Finally, we saw differences in left *vs* right side in certain regions. Left side, not the right side, shows significant MD changes in anterior limb of internal capsule and external capsule, FA changes in posterior corona radiata, RD changes in external capsule. On the other hand, right side shows significant FA changes in uncinate, RD changes in posterior limb of internal capsule. Overall, we has studied DTI changes in all the major white matter tracts in the central nervous system. It shows limbic structures related to memory and emotions have significant changes in AD in comparison to control, consistent with some other studies, but not corticospinal tract that is involved in primary motor functions. Left and right sides of these structures of the brain are affected with differences in certain tracts. There might be gender differences in terms of the severity of these DTI changes, indicated by MD.

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Poster

P213. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

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Program #/Poster #: P213.02

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

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Title: White matter hyper-intensities and cognitive decline in APOE4 carriers

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Abstract: Purpose: Subcortical white matter sessions, assumed to be on ischemic bases, are common in older subjects and correlate with cognitive decline in aging and during AD progression. Less pronounced than in the hippocampus, our team observed a trend of early blood-brain barrier (BBB) breakdown in the white matter of patients with mild cognitive decline (MCI), raising the possibility that BBB breakdown and not ischemia is an early determinant of white matter hyper-intensity. Indeed, ApoE4 gene is a major genetic risk factor for Alzheimer's disease. Here, we investigated the white matter hyper-intensities (WMH) burden in addition to ApoE4 gene and cognitive performances. 135 patients were included in this study with available high-resolution T2w-FLAIR MRI. **Methods**: Out of 135 subjects, 64 were ApoE4 non-carriers (3/3) and 71 ApoE4 carriers (3/4+4/4). In ApoE4 non-carriers, 54 were cognitively normal and 10 were showing Clinical Dementia Rating (CDR®) score of 0.5. In ApoE4 carriers, 60 were cognitively normal and 11 were CDR 0.5. WMH burden (reported in mL) was estimated on a T2w FLAIR-MRI high-resolution images (1x1x1.2mm^3). **Results**: Results suggest that ApoE4 carriers demonstrates larger WMH burden with age in cognitively normal patients. Moreover, study findings suggest that WMH burden is not significantly different in ApoE4 non-carrier and carriers. As supported by the results, patients who manifest cognitive decline, their WMH burden is larger compared to cognitively normal patients. **Conclusions**: High-resolution MRI is a powerful tool to assess white matter hyper-intensities and its relationship to BBB integrity. To understand BBB permeability contribution in WMH progression, we will next analyze DCE-MRI extracted Ktrans values within WMH burden as well as in normal-appearing white matter.

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Poster

P213. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

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Program #/Poster #: P213.03

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

Support: CIHR
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Title: Involvement of lipoxygenase mediators in executive function and microstructural white matter integrity in patients with Alzheimer's disease and subcortical ischemic vascular disease

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Abstract: Hydroxyoctadecadienoic acid metabolites (9-HODE and 13-HODE) have anti-inflammatory properties but their clinical associations with Alzheimer's disease (AD) and subcortical ischemic vascular disease (SIVD) have not been established. We aim to examine these metabolites with respect to cognition and microstructural white matter integrity.
Patients from memory and stroke prevention clinics (n=69) were stratified based on SIVD (minimal vs. abundant white matter hyperintensities [WMH] based on 3.0T structural MRI), and AD (clinical diagnosis). Oxylipins were extracted from serum using solid phase extraction and their concentrations measured by ultra high-performance liquid chromatography mass spectrometry. Microstructural tissue integrity was examined as fractional anisotropy (FA) and mean diffusivity (MD) using diffusion tensor imaging. Executive function was measured by the Trail Making Test B (TMT B).
Using a linear regression model controlling for age, sex, AD, and SIVD, both 9-HODE and 13-HODE were associated with better performance on the TMT B (β=0.279 p=0.008 and β=0.276 p=0.010) but had no significant associations with verbal memory measured by long delay free and cued recall in the California Verbal Learning Test. 9-HODE was associated with higher FA in normal appearing white matter in the left superior frontal (β=0.284 p=0.023), right middle frontal (β=0.243 p=0.044), and 9-HODE and 13-HODE in the left inferior frontal region (β=0.274 p=0.026 and β=0.283 p=0.022). In these same regions, normal appearing white matter FA was associated with better performance on the TMT B (β=0.255 p=0.027, β=0.277 p=0.020, and β=0.322 p=0.005). 13-HODE was associated with higher FA in deep WMH in the left superior frontal (β=0.465 p=0.004) and 9-HODE and 13-HODE in right middle inferior frontal regions (β=0.744 p=0.002 and β=0.570 p=0.021). 9-HODE was associated with lower MD in deep WMH in the left middle medial frontal (β=-0.506 p=0.040) region. 9-HODE and 13-HODE were associated with less atrophy in the left temporal (β=-0.207 p=0.024 and β=-0.240 p=0.009) and right temporal (β=-0.255 p=0.016 and β=-0.239 p=0.011) regions. In patients with only SIVD, 9-HODE and 13-HODE were associated with smaller periventricular WMH volume (β=-0.524 p=0.043 and β=-0.594 p=0.013).
Anti-inflammatory oxylipins were associated with better performance on the TMT B, smaller vascular brain lesion volumes and lesion microstructural characteristics, less atrophy, and preserved white matter microstructural integrity in frontal regions. Together, these associations suggest a neuroprotective role of 9-HODE and 13-HODE on the small vessels and executive function.

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Poster

P213. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

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

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Title: Postmortem neocortical ^{3}H-PiB binding and levels of unmodified and pyroglutamate Aβ in Down syndrome and sporadic Alzheimer’s disease

Authors: *V. PIVTORAIKO[1], T. RACIC[1], E. E. ABRAHAMSON[1,3], V. L. VILLEMAGNE[2], B. L. HANDEN[2], I. T. LOTT[4], E. HEAD[5], M. D. IKONOMOVIC[1,3];
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Abstract: Individuals with Down syndrome (DS) have a genetic predisposition for amyloid-β (Aβ) overproduction and earlier onset of Aβ deposits compared to sporadic late-onset Alzheimer’s disease (AD). Positron emission tomography (PET) with Pittsburgh Compound-B (PiB) detects fibrillar Aβ pathology in living people with DS and AD, but its relationship with heterogeneous Aβ forms aggregated within amyloid deposits is not well understood. We performed quantitative in-vitro ^{3}H-PiB binding assays and enzyme-linked immunosorbent assays of fibrillar (insoluble) unmodified Aβ40 and Aβ42 forms and N-terminus truncated and pyroglutamate-modified AβNpE3-40 and AβNpE3-42 forms in postmortem frontal cortex and precuneus samples from 18 DS cases aged 43-63 years and 17 late-onset AD cases aged 62-99 years. Both diagnostic groups had advanced stages of amyloid plaque pathology, and DS cases had, on average, more severe vascular amyloid pathology (cerebral amyloid angiopathy, CAA). Compared to the AD group, the DS group had higher levels of Aβ40 and AβNpE3-40, while the two groups did not differ by Aβ42 and AβNpE3-42 levels, in both the frontal cortex and precuneus. This resulted in lower ratios of Aβ42/Aβ40 and AβNpE3-42/AβNpE3-40 in the DS

group compared to the AD group, in both cortical regions. Correlations of Aβ42/Aβ40 and AβNpE3-42/AβNpE3-40 ratios with CAA severity were strong in DS cases, and weak in AD cases. The two diagnostic groups did not differ significantly by ^{3}H-PiB binding levels in the frontal cortex or in the precuneus. These results demonstrate that compared to late-onset AD cases, adult DS individuals with similar burden of cortical Aβ plaques have a preponderance of both pyroglutamate-modified AβNpE3-40 and unmodified Aβ40 forms which are associated with greater CAA pathology. Despite the distinct molecular profile of Aβ forms and greater vascular amyloidosis in DS cases, cortical ^{3}H-PiB binding does not distinguish between diagnostic groups that are at an advanced level of amyloid plaque pathology. These results underscore the need for development of CAA-selective PET radiopharmaceuticals in order to detect and track the progression of cerebral vascular amyloid deposits in relation to Aβ plaques at the earliest pathology stages in individuals with DS.

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Poster

P213. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

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Program #/Poster #: P213.05

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

Support: NIH Grant R01 AG 046266
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Title: Markers of brain aging in cognitively characterized marmosets (Callithrix jaccus)

Authors: *C. FREIRE-COBO[1,2], M. VARGHESE[1,2], E. ROTHWELL[3], M. EDWARDS[3], W. G. M. JANSSEN[1], A. LACREUSE[3], P. R. HOF[1,2,4];
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Abstract: The common marmoset (*Callithrix jacchus*), a small nonhuman primate with highly developed social and cognitive abilities that lives for about 10 years and shows AD-like neuropathology in old age, constitutes an important primate model for longitudinal studies of neurocognitive aging. Some of the hallmarks of AD pathology, including amyloid beta and tau deposition, astrocytic proliferation and microglial atrophy, have been described in the brains of old marmosets. However, the relationships between these pathological hallmarks and cognitive function have not been established in this species. In addition, the loss of dendritic spines or

changes to their morphology, which have been proposed to mediate age-related cognitive decline in macaques has not been assessed in marmosets. We analyzed brain tissues from 16 male and female marmosets (7-9 years old) that had been behaviorally characterized in a 4-year longitudinal study as they transitioned to old age (Rothwell et al., in revision). Analysis of cognitive trajectories, focused on reversal learning, indicated that some monkeys maintained or even improved cognitive performance across aging, while others showed a decline starting around age 8. The analysis of brain tissues was focused on the dorsolateral prefrontal cortex (dlPFC, layer III of area 8b/9) and the CA1 field of the hippocampus, for their relevance reported in cognitive studies in NHP. We targeted several neural and glial markers in a series of immunofluorescence assays including: glial fibrillary acidic protein (GFAP) and ionized calcium-binding adaptor molecule 1 (Iba1) in astrocytes and microglia cells respectively; cluster of Differentiation (CD68) for identifying lysosome activation; anti-beta amyloid antibody (MOAB-2) for analyzing soluble and insoluble forms of amyloid deposits; anti-Collagen IV for brain blood vessels and Neurotrace as a neuronal pan marker. For the morphological analysis of spines we performed iontophoretic dye-filling of pyramidal neurons, followed by confocal imaging of their dendritic segments and three-dimensional reconstruction of spines. The analysis revealed
highly variable degrees of amyloid beta neuropathology among individuals. Glial changes included changes on branching and soma size in astrocytes, increased microglia activation and cell atrophy. We also identified differences in spine vulnerability during aging in marmosets. Our preliminary analysis suggests that some marmosets show an accelerated or pathological aging profile characterized by a higher degree of amyloid beta deposition, astrogliosis, morphologic alterations in microglia and loss of dendritic spines.

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Poster

P213. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

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Program #/Poster #: P213.06

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

Support: NIH Grant RF1 AG072637
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Title: Total hippocampal and CA4-DG volumes are reduced in Alzheimer's Disease compared to Vascular Cognitive Impairment

Authors: *I. KWAK[1], D. HOBBS[2], C. A. RAJI[2];
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Abstract: Total hippocampal and CA4-DG volumes are reduced in Alzheimer's Disease compared to Vascular Cognitive Impairment

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Background: Hippocampal volume abnormalities are detailed in multiple neurocognitive disorders. Hippocampal subfields may add value in clinically delineating these conditions. Manual segmentation remains the gold standard but is limited by time and inter-operator variability. We therefore utilized the automated segmentation tool, volBrain's HIPS pipeline, to analyze hippocampal subfield volumes in Alzheimer disease (AD) and diagnoses of mixed dementia.

Methods: A total of 134 participants (ages 56-94) were drawn from the Open Access Series of Imaging Studies 4 (OASIS-4) and classified into 5 diagnostic groups: AD/Dementia (AD/D, n = 50), AD/Vascular Dementia (AD/VD, n = 38), Frontotemporal Dementia (FTD, n = 20), Parkinson's Disease (PD, n = 10), and Vascular Cognitive Impairment (VCI, n = 16). T1- and T2-weighted brain MRI scans collected on a 3T scanner were processed through volBrain's HIPS pipeline, resulting in 5 subfield volumes: Cornu Ammonis (CA) 1, CA2-CA3, CA4-Dentate Gyrus (DG), Strata Radiatum/Lacunosum/Moleculare (SR-SL-SM), and Subiculum. Analysis of covariance (ANCOVA) were used to compare subfield volumes between diagnostic groups when controlling for age, sex, and intracranial volume (ICV). Results were corrected for multiple comparisons ($p < 0.005$).

Results: Subfield volumes varied by diagnosis with medium to large effect sizes. Total hippocampal volume is lower in AD/D ($p < 0.001$, $d = 1.37$) and AD/VD ($p < 0.005$, $d = 1.06$) when compared to VCI. CA4-DG volume is lower in AD/D ($p < 0.005$, $d = 1.05$) and AD/VD ($p < 0.005$, $d = 1.12$) when compared to VCI.

Conclusions: Decreased hippocampal volume is a core biomarker of AD and is used to stage progression of AD pathology. The subgranular zone of the dentate gyrus is a critical site of neurogenesis that is impaired in AD. We found that both total hippocampal and CA4-DG volumes were significantly lower in AD when compared to VCI. Further study will validate the use of volBrain and other automated segmentation protocols as a potential diagnostic tool for neurocognitive disorders.

Disclosures: I. Kwak: None. **D. Hobbs:** None. **C.A. Raji:** None.

Poster

P213. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

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Title: Modeling multimodal brain-predicted age in early symptomatic and preclinical Alzheimer disease

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Abstract: Background: The “brain-predicted age” approach quantifies apparent brain age compared to normative trajectories of neuroimaging features. Elevated brain-predicted age has been observed in symptomatic Alzheimer disease (AD), but has not been evaluated in preclinical AD. Prior studies have typically modeled brain age differences with volumetric magnetic resonance imaging (MRI), but other modalities, including resting-state functional connectivity (FC) and multimodal models, have only been more recently explored. In the present study, we modeled brain age using unimodal FC and volumetric data, as well as multimodal models, to compare the models’ sensitivity to symptomatic and preclinical AD, and further, test relationships with cognitive performance.
Methods: We measured regional brain volumes and FC in 493 control participants (cognitively normal, amyloid-negative, 18-89 years old). We trained three Gaussian process regression models to predict age from (1) FC, (2) volumetric, and (3) multimodal features. We applied the trained models to separate sets of 153 older adult controls, 141 preclinical AD (cognitively normal, amyloid-positive), and 154 symptomatic AD participants to test for group differences. Preclinical AD pathology was assessed using PET (Centiloid) and CSF (amyloid β 42 [Aβ42], phosphorylated tau 181 [pTau], pTau/Aβ42 ratio). Cognition was assessed using a composite of neuropsychological tests across multiple domains.
Results: All models successfully predicted age in the control training set, with the multimodal model (mean absolute error [MAE] = 5.3 years) outperforming the FC (MAE = 8.3) and volumetric models (MAE = 6.0). In all three models, the brain age gap (BAG) was significantly elevated in symptomatic AD compared to cognitively normal controls (p’s < .003). In the FC and multimodal models, BAG was marginally reduced in preclinical AD participants with elevated pTau (p’s < .06). In symptomatic AD, lower global cognitive composite scores were associated with elevated volumetric and multimodal BAGs.
Discussion: Both FC and volumetric estimates of brain age are sensitive to symptomatic AD, consistent with previous reports. Additionally, FC-based brain age may exhibit a biphasic

response to preclinical AD pathology while volumetric brain age may be sensitive to cognitive decline in the symptomatic stage. Multimodal estimates of brain age capture each of these modality-specific relationships, and further, improve sensitivity to healthy age differences. Hence, multimodal neuroimaging models may be useful in staging early symptomatic and preclinical AD.

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Poster

P213. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P213.08

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

Support: CIHR Grant MOP-125915

Title: Visuomotor task performance declines in older adults with a genetic (APOE e4) risk for Alzheimer's disease: Grey and white matter changes

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Abstract: Background: Cognitive-motor integration (CMI) is a type of visuomotor integration that involves concurrent thought and action and requires the interaction of large networks in the brain. Previous findings have shown that CMI performance is impaired in individuals with specific dementia risk factors (family history of dementia and presence of the APOE e4 allele) in advance of any cognitive impairments. These findings suggest that CMI impairments are associated with early dementia-related brain changes. The objectives of the current research study are to assess the relationship between grey and white matter changes and CMI performance, as well as the impacts of dementia family history, sex, and APOE status. Methods: Participants included right-handed older adults with a family history of dementia (n=25, 12 female) or no family history of dementia (n=24, 12 female). APOE genotyping was determined from salivary measures. Participants were tested on four visuomotor tasks where reach and gaze were increasingly spatially dissociated using two linked touchscreens. These included a standard condition requiring direct interaction with visual targets, and three dissociated non-standard conditions requiring CMI (visual feedback reversal, plane-change, and plane-change + feedback reversal). T1-weighted anatomical scans were used to quantify grey matter volume and thickness in brain regions important for CMI. Diffusion tensor imaging (DTI) measures of fractional anisotropy, mean diffusivity, radial diffusivity, and axial diffusivity were collected to look at white matter integrity. Results: Males had smaller left inferior parietal lobule thickness compared

to females ($F_{1,43} = 10.15$, $p < 0.01$). Our previous behavioural results demonstrated that males performed worse on the feedback reversal condition compared to females, and research has shown that the inferior parietal lobule is important for visually-guided reaching. Changes in DTI measures in several major white matter tracts were a significant predictor of poorer CMI performance only in APOE e4 carriers. The interaction effect between diffusion measures and APOE status on CMI performance were all significant at $p < 0.05$. These data support our hypothesis that disruptions in CMI performance are associated with identifiable brain alterations early in disease progression. Conclusion: The preliminary findings provide insight into the impact of AD pathology, genetics, and sex on neural networks underlying complex visuomotor transformations, and demonstrate that the CMI paradigm discussed in this study may be used as a non-invasive, easily accessible assessment tool for dementia risk.

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Poster

P213. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

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Program #/Poster #: P213.09

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

Support: Center for Biomedical Neuroscience (CBN) 2020-21

Title: Preclinical Imaging of Microtubules in Animal Models of Alzheimer’s Disease

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Abstract: An ideal neuroimaging PET tracer should have high signal to noise ratio in pathologically relevant brain regions, be able to monitor progression or regression of disease in correlation with clinical findings, and have utility for guiding treatment and new drug development. Currently, no such PET imaging agent is available for neurodegenerative disorders. For Alzheimer’s disease, clinical diagnosis is frequently not well correlated to imaging findings with Amyloid beta and current tau tracers. Among the disease-specific abnormal proteins reported, abnormalities in the dynamics of microtubules have been identified as a validated biomarker for neurodegenerative disorders and its loss in Alzheimer’s disease and other neurodegenerative disorders are in correlation with neurodegeneration. At present, the blood brain barrier-penetrating PET tracer, [^{11}C]MPC-6827, which we have developed is the

only PET tracer available for in vivo imaging of microtubules in brain. The central hypothesize is that microtubule targeted PET tracer will provide a higher signal to noise ratio, compared with tau, and Amyloid beta tracers, since the presence of tau and amyloid beta proteins likely represents only a subset of microtubule associated abnormalities present in neurodegeneration. The hypothesis was tested via head-to-head comparison of [^{11}C]MPC-6827 with tau PET tracer, [^{18}F]MK-6240 in transgenic tau mouse models of hTau, PS19 and age matched controls (n=4 per group). Preclinical imaging was performed Siemens Focus 220 microPET scanner for 30-minute dynamic acquisitions with the above mentioned two PET tracers. PET image analyses showed reduced whole brain, hippocampus and prefrontal cortex uptake of [^{11}C]MPC-6827 in hTau and PS19 mouse groups, whereas, [^{18}F]MK-6240 exhibit a modest higher binding in corresponding mouse models than controls. [^{11}C]MPC-6827 has higher standardized uptake value and effect size compared to [^{18}F]MK-6240 binding in both transgenic mouse models. This pilot preclinical imaging studies show a trend of reduced binding of the microtubule targeted PET ligand, [^{11}C]MPC-6827 in whole brain and brain regions of hTau and PS19 transgenic mouse models compared to control group and is inversely correlated with [^{18}F]MK-6240 binding. This suggests a likelihood of a higher binding difference of [^{11}C]MPC-6827 than tau tracer in human Alzheimer's disease compared to control subjects. Therefore, [^{11}C]MPC-6827 can be used as a potential PET tracer for human brain imaging of Alzheimer's disease and other neurodegenerative diseases.

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Poster

P213. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

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Program #/Poster #: P213.10

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

Support: NIH T32 AG057468
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Title: Age-related changes in mice with a single copy insertion of human App with familial Alzheimer's disease mutations using diffusion tensor imaging

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Abstract: Alzheimer's disease is a progressive neurodegenerative disease. The familial form (FAD) is caused by mutations in amyloid precursor protein (APP) or presenilin-1,2. While many mouse models expressing FAD-linked mutant forms have been produced, transgenic overexpression of APP raised concerns of severe artifacts. The *App*$^{NL\text{-}G\text{-}F/NL\text{-}G\text{-}F}$ (APPKI) model has a single copy knock-in of human *App* with the Swedish, Arctic, and Beyreuther/Iberian mutations, which may offer improved clinical translation. In this study, we utilized diffusion tensor imaging in wild-type and APPKI mice in young, middle, and late age groups to observe how FAD-linked APP mutations alter white matter architecture to identify potential early biomarkers of AD. A 9.4 T Agilent MRI system was used to scan wild-type and APPKI female mice *in vivo*. A T2-weighted sequence followed by 12-direction diffusion-weighted sequence was then performed. The tensor model was fit to the diffusion-weighted data using FSL's dtifit. Atlas labels were registered to each individual's subject space. The mean fractional anisotropy (FA) was then measured in each region of interest (ROI) for each subject as a proxy of white matter integrity. A three-way mixed ANOVA was then used to model the data, using age group and genotype as between-subjects measures and brain ROIs as a repeated measure. We observed statistically significant main effect of genotype, as well as statistically significant interactions between age group and brain region and between genotype and brain region. The largest reductions in FA were observed in the hippocampus and cortex as well as the anterior commissure and the hypothalamus. Together, these results suggest that microstructural impairments take place in these specific regions in the APPKI mice. Furthermore, these changes occur between 10- and 17-months of age, which is approximately middle- to late-aged in humans, suggesting that this may represent a time window with early macroscale white matter changes that are potential targets for therapeutic investigation.

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Poster

P213. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

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Program #/Poster #: P213.11

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

Support: R01AG067228

Title: Investigating temporal dynamics of vascular changes in Alzheimer's disease mouse model using optical coherence tomography

Authors: *J.-H. LEE[1], T. YANG[2], J. NIE[1], J. LEE[1];
[1]Brown Univ., Providence, RI; [2]Korea Univ., Seoul, Korea, Republic of

Abstract: Vascular dysfunction shows its high prevalence in Alzheimer's disease (AD), but much is still unknown for vascular alterations in relation to key AD pathological changes such as cognitive impairment, amyloid beta plaque accumulation, and neuronal loss. In this study, we demonstrated a label-free spectral-domain optical coherence tomography (SD-OCT) imaging to thoroughly observe temporal dynamics of vascular alterations *in vivo* using AD transgenic mouse model (3xTg-AD). We acquired OCT angiogram (OCTA) and Doppler OCT (DOCT) datasets to quantify relative changes in diameter and blood flows for pial vessels and penetrating arterioles and venules. The OCT imaging started at 11 weeks of age (WOA) then repeated every 4 weeks until the mice reach 57 WOA for mice groups. To confirm cognitive impairment, we conducted novel object location test as a behavioral test repeated every 4 weeks also. We performed Bielschowsky silver staining as post-mortem immunohistochemistry for validating amyloid burden in hippocampal and cortical region. As a result, we found the cognitive decline occurring at 40 WOA whereas the diameter change of pial vessels occurred much earlier at around 20 WOA for AD mice group. Similar to the tendency found in OCTA analysis, for DOCT, the diameter change for penetrating arterioles in AD group showed early change at around 13 WOA, whereas the change in penetrating venules seemed trivial. Interestingly, the change (from 11 to 57 WOA) in mean diameter for penetrating arterioles (3.6 μm) was about 9-fold compared to that of penetrating venules (0.4 μm) in AD group. For blood flow change, penetrating arterioles and venules both showed the decaying tendency with the rapid change occurred at early stage before 20 WOA for AD group. To determine whether vascular alteration affects cognitive function, we used 3 different drugs that are known to have vasodilating effect: one vasodilating drug, nicorandil, and one FDA-approved AD drug, memantine. These drugs were administered via drinking water starting after 15 WOA. We found no sign of cognitive decline and no significant diameter change in pial vessels from these two drug-administered mice groups, similar to that of wild-type (WT) group. For the change in diameter and blood flows for penetrating arterioles and venules, these two drug-administered groups again showed the similar tendency to that of WT group, implying that the vasodilating effect seems to be correlated with cognitive impairment. Overall, our finding supports that understanding the temporal dynamics of vascular alterations in relation to cognitive decline could serve as a basis for translational research aiming toward early diagnosis of AD.

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Poster

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

Support: NIH/NIA Grant R01 AG55132
Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu)

Title: Predictive models of cognitive scores in aMCI using functional connectomics

Authors: *D. J. NOWLING, G. C. WARNER, N. BUSTOS, K. L. BARLIS, J. E. JOSEPH; Neurosci., Med. Univ. of South Carolina (MU Neurosci. Inst. - Graduate), Charleston, SC

Abstract: Alzheimer's Disease (AD) is a leading cause of cognitive decline and death. Mapping its prodromal stage amnestic Mild Cognitive Decline (aMCI) is critical to risk identification and intervention, and functional MRI (fMRI) connectomics can help identify networks important to specific cognitive domains in aMCI. Many network studies of aMCI only focus on a few or one cognitive domain at a time. To create a more complete picture of network profiles important in aMCI this exploratory study uses graph theory to build and compare predictive graph theory (GT) based models of 7 cognitive domains. 90 male and female human subject resting fMRI scans from the Alzheimer's Disease Neuroimaging Initiative with an average age of 68.18 years were used. Datasets were split into subgroups based on available cognitive score: Mini-Mental State Examination (MMSE) n = 90, Logical immediate (LIMM) and delayed (LDEL) n = 88, Trails A (TRAA) and B (TRAB) n = 72, Multilingual Naming Test (MINT) n = 52, and Rey Auditory Verbal Learning Test (RAVLT) immediate n = 51. GT measures of strength* (STR), eigenvector centrality (EVC), and clustering coefficient (CC) were calculated from time series of 276 a priori nodes. Dimension reduction involved using Principal Components Analysis (top 25 variance explaining components) or highest ranking 25 nodes for each GT measure, which were used as predictors of cognitive score in SPSS Automatic Linear Modeling (42 models were tested). The best model for each cognitive score was defined as the model that yielded at least 50% significant models across 20 cross-validations. The network profile for LIMM had auditory and post central gyrus nodes based on CC, RAVLT had postcentral sensory and posterior cingulate cortex nodes based on CC, TRAA had precuneus, middle frontal, fusiform, and paracingulate gyrus nodes based on EVC, and TRAB had posterior cingulate, entorhinal, superior parietal, frontal pole, and temporooccipital cortex nodes based on EVC. These results show that different cognitive domains in aMCI not only have different important anatomical features, but they also vary in terms of GT properties, indicating different aspects of functional organization and communication affect different cognitive domains in the same network. These preliminary findings warrant further work into developing a comprehensive map of aMCI network models to identify both interactions and differences in the network dynamics of cognitive processes disrupted during prodromal AD, as a first step toward creating clinically meaningful phenotypes.

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Poster

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Title: Bold fmri mean signal changes in healthy aging and dementia: a preliminary study

Authors: *M. P. MCAVOY, M. S. GOYAL, B. A. GORDON, B. M. ANCES, J. C. MORRIS, A. G. VLASSENKO, T. L. S. BENZINGER;
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Abstract: BOLD resting state fMRI is enjoying great popularity with its inclusion in many large neuroimaging studies. Analyses have been largely confined to the temporal fluctuations of the BOLD time series, while the mean signal of these fluctuations has been virtually ignored. Here we show that changes in mean BOLD signal distinguished between groups of healthy aging adults, individuals with significant PET amyloid deposition and those with Alzheimer disease (AD). Cohorts included 117 cognitively normal, PET amyloid negative individuals (i.e. “normal”, age 49 - 91.3), 55 cognitively normal, PET amyloid positive individuals (i.e. “preclinical”, age 57 - 87.4) and 30 PET amyloid positive, AD symptomatic individuals (i.e. “symptomatic”, age 62 - 89). All data were collected on a 3T Siemens Biograph mMR PET-MR system and included two six minute eyes open resting state BOLD scans (TR = 2.2s). The subjects’ BOLD mean signals were input to a group ANOVA implemented via a general linear model that included age variables centered for each cohort. In an analysis of 84 Freesurfer gray matter regions, statistically significant differences between the three cohorts after Bonferroni correction ($p = 0.05/84 = 0.0006$) were found in the caudate bilaterally and right caudal anterior cingulate. These cohort effects were further decomposed with three post hoc paired comparisons: 1) normal vs preclinical, 2) normal vs symptomatic and 3) preclinical vs symptomatic, which were Bonferroni corrected ($p = 0.05/3 = 0.017$). Left caudate was found to be affected by cognitive decline and amyloid deposition, while right caudate was affected only by cognitive decline and the right caudal anterior cingulate was affected only by amyloid deposition. In an age analysis, all cohorts in all regions showed a trend for decreasing BOLD mean signal with age, however this only reached statistical significance for the normal cohort in the right caudate ($P < 0.05$). Our results suggest that the mean BOLD signal, whose source requires further

investigation though is likely in part related to oxygen metabolism, might be a valuable measure of resting physiologic changes of the brain in healthy aging and AD.

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Poster

P213. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P213.14

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

Title: Harmonizing MRI using Attention-Guided Generative Adversarial Networks improves Alzheimer's Disease Classification Performance

Authors: *S. SINHA[1], S. I. THOMOPOULOS[2], P. LAM[3], A. MUIR[3], P. M. THOMPSON[4]; [1]USC, San Jose, CA; [2]USC, Marina del Rey, CA; [3]USC, Los Angeles, CA; [4]Stevens Inst. for Neuroimaging & Informatics, Univ. of Southern California (USC), Marina Del Rey, CA

Abstract: Alzheimer's disease (AD) is characterized by irreversible memory loss and declining performance across multiple cognitive domains, and is the most common form of dementia accounting for over half of dementia cases worldwide. Magnetic resonance imaging (MRI) can be used to detect subtle and diffuse brain structural changes in AD, offering opportunities to develop deep learning methods to detect AD. However, scanners and imaging protocols differ widely, and image classification models need to be tolerant to shifts in image characteristics. Here we evaluated a new deep learning approach for domain adaptation of MRI scans from various sources to test whether this step assisted AD classification performance using a convolutional neural network model. To achieve MRI harmonization, we adapted an Attention Guided Generative Adversarial Network as defined in (Tang et al. 1) and tested it on 3 public MRI datasets: ADNI, AIBL and OASIS. The Attention-GAN architecture optimized an objective function including adversarial loss, cycle-consistency loss, pixel loss and attention loss. Following domain adaptation, we performed AD classification with a well-established 2D AlexNet CNN (Krizhevsky et al. 2) adapted to handle brain MRI. MRI harmonization improved AD classification accuracies by more than 9% (reaching 84 % accuracy) as compared to accuracies using non-harmonized images from the 3 datasets. As AIBL only included AD cases, we even tested our hypothesis using just ADNI and OASIS and observed a 2-3% increase in accuracy. The smaller increase in accuracy may reflect the lower number of OASIS subjects and a greater domain shift with ADNI dominating the dataset. To increase efficiency, we tested

optimizing the position of the axial, sagittal and coronal 2D slice subsets used for classification. MRI harmonization increased AD classification accuracy irrespective of slicing positions. Figure 1 shows an example of the MRI harmonization results.
1. https://arxiv.org/abs/1903.12296 2. ImageNet Classification with Deep Convolutional Neural Networks

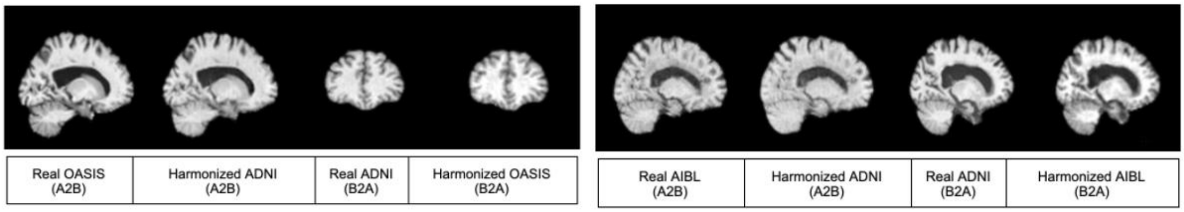


Figure 1. Domain adaptation from OASIS to ADNI and from AIBL to ADNI.

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Poster

P213. Clinical and Pre-Clinical Imaging Studies in Alzheimer's Disease

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Program #/Poster #: P213.15

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

Title: Ambulatory EEG sleep monitoring in Alzheimer disease A Pilot Study

Authors: *B. KENT[1], S. CARLUCCI[2], A. CASCIOLA[2], M. CHEN[2], P. SLACK[2], A. KAZEMI[4], M. MIRIAN[2], S. STAGER[5], J. VALERIO[2], M. MCKEOWN[2], H. FELDMAN[6], H. B. NYGAARD[3];
[1]Simon Fraser Univ., Burnaby, BC, Canada; [3]NEUROLOGY, [2]Univ. of British Columbia, Vancouver, BC, Canada; [4]Univ. of California, Davis, Davis, CA; [5]McMaster Univ., Hamilton, ON, Canada; [6]Univ. of Califrnia, San Diego, La Jolla, CA

Abstract: Sleep disturbances are common in Alzheimer’s disease (AD), with estimates of prevalence as high as 65% across AD and Mild Cognitive Impairment (MCI). A major limitation to a formal, qualitative assessment of sleep in these populations is the requirement for inpatient polysomnography (PSG), which is often not well tolerated in patients with dementia. We have recently developed a deep learning model to reliably analyze lower quality EEG data obtained from a simple, 2-lead EEG headband. This methodology allows for ambulatory assessment of sleep, with the potential for more widespread sleep staging particularly in vulnerable populations. Here we present a pilot study of ambulatory EEG sleep assessment in patients with

AD and non-demented control subjects. A total of 20 AD patient and 17 age-matched, non-demented control subjects underwent ambulatory EEG. Recording from 4 AD patients and 4 control subjects were discarded due to corrupt data. Each subject wore the headband for up to 3 nights. A deep learning model was previously developed by our group in healthy volunteers undergoing both PSG as well as headband EEG recordings. The training model contained three convolutional layers (8 filters) followed by two LSTM layers (64 units). EEG features in the following categories were extracted: a) frequency domain; b) time domain; c) higher-order statistical analysis (HOSA)-base, and (d) wavelet based. In order to increase the validity of the predictions, we generated 12 models based on a subset of the original PSG validation study. When a majority of models did not agree on a prediction, the data were discarded. AD patients exhbited less N3 sleep than control subjects. No differences were found in N2 or REM sleep stages. Actigraphy supplementing the EEG will be presented. We show that ambulatory EEG is feasible in patients with AD. Moreover, using a deep learning model, relatively low quality, 2-lead EEG headband data can stage sleep in an ambulatory setting. Similar to studies using PSG, we find that N3 sleep is reduced in patients with AD. Our findings suggest that ambulatory EEG is a promising approach for future studies addressing sleep quality in patients with dementia.

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Poster

P219. Roles of Amyloid Beta in Cognition and Behavior

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Program #/Poster #: P219.01

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

Title: Examining behavior of a double transgenic (APP/tau) mouse model of Alzheimer's disease on zinc and copper supplementation

Authors: *T. GERVASE, A. BOOTH, S. NEFF, R. HOYOS JUSTINIANO, M. S. LAMARCHE, L. E. ALLEN, J. M. FLINN;
George Mason Univ., Fairfax, VA

Abstract: Alzheimer’s Disease (AD) is an extremely prevalent neurocognitive disorder influencing both brain and behavior. AD is neurologically characterized by two major components; amyloid plaques and neurofibrillary tangles. Although many mouse models contain only one of these components, our lab recently developed a dual transgenic (Tg) mouse which contains both amyloid and tau via a J20 (hAPP) and P301L (Tau) cross. As this mouse model does not contain mutations on the Presenlin-1 (PS1), one of the most common causes of early-onset AD, this mouse models late-onset AD. Additionally, prior research in our lab indicated an

improper balance of biological metals such as Zinc (Zn) may exacerbate AD-pathology and behavioral deficits. However, as Zn has been shown to decrease Copper (Cu) levels it is unclear whether these deficits were due to an excess of Zn or an induced copper deficiency. To assess this WT (n=42) and Tg (n=40) mice were given either lab water (n=27), water supplemented with 10 ppm Zn (n=27), or water supplemented with 10 ppm Zn and 2.5 ppm Cu (n=28). Hippocampal damage is one of the first regions affected by AD and is known to play a large role in spatial memory. Previous research in our lab used the Barnes Maze to assess spatial memory deficits, however, in our current study we examined spatial memory via the Morris Water Maze (MWM) to further examine this mouse model. Mice were tested at 3.5 months of age with latency to find the platform being assessed. Using ANOVA our findings indicated that Tg mice showed significant spatial memory impairments compared to WT mice, ($p < .001$). Although there were no differences between groups based on metal supplementation this matches previous data in our lab which did not find differences at 4 months possible because the genotype effects were so strong. Results will be reanalyzed at 7-months of age including circadian activity, burrowing, and nesting. Additionally, brains will be analyzed using Western Blots for proteins of interest. Our findings provide additional evidence that this new Tg mouse is able to properly model the behavioral deficits seen in late-onset AD without PS1.

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Poster

P219. Roles of Amyloid Beta in Cognition and Behavior

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

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R01 MH099073 (J.J.K.)
2019R1F1A1063005 (J-H.J.)

Title: Naturalistic risky decision-making in a mouse model of Alzheimer's disease

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Abstract: While considerable Alzheimer’s disease (AD) research has focused on destructive loss of memory, much less is known about the impact of AD on other cognitive functions, such as

risky decision-making which plays pervasive roles in daily life. Here, we examined whether the ability to discern safety-danger boundaries and make optimal foraging decisions was altered in a mouse model of AD. Five familial AD (5XFAD) transgenic mice (4-8 mos old, both sexes) that overexpress amyloid precursor protein and presenilin 1 were tested in a naturalistic 'approach food-avoid predator' paradigm (Choi & Kim, 2010). Animals underwent nest habituation, baseline foraging, and predator testing. During the test trials, as the mouse approached a food pellet, placed 38.1, 25.4, and 12.7 cm from the nest, a taxidermied weasel on wheels was automatically activated to surge 30 cm distance (60 cm/s). Mice were given 3 min to procure the pellet (one trial each distance) per daily session. There were no reliable differences across sex and age groups, and thus mice were pooled in each genotype. In response to the surging weasel, both wildtype (WT) and 5XFAD mice promptly fled into the nest, and the latency to procure pellets located at 38.1 cm increased significantly. However, 5XFAD animals' latency to procure pellets placed at 25.4 cm did not differ from the baseline foraging latency, suggesting precarious decisions. Given that animals naturally switch their foraging strategies from high predator to low predator density area (Stephens et al., 2007), we then tested whether 5XFAD mice were able to adjust their foraging in 'conditional threat' situations. To do so, grain-based and chocolate-flavored pellets were placed in separate arms of a T-shaped maze. After mice have freely chosen their preferred and non-preferred pellets, on the test trials, every time the animal near the preferred pellet, the predator surged forward. While the WTs appropriately switched their choice from the preferred to non-preferred pellets, the 5XFADs continued to choose their preferred pellets, indicating impairment in adjusting their foraging behavior. Lastly, when animals were subjected to contextual fear conditioning to assess fear responses to physical pain, both WTs and 5XFADs exhibited comparable postshock freezing and contextual freezing the next day. In 5XFAD (but not WT) animals, high levels of amyloid β deposition were found in the prelimbic cortex, hippocampal CA1, and amygdala, regions implicated in risky decision-making in humans and rodents. These results suggest that risky decision-making is impaired in AD mice and may be a more sensitive behavioral phenotype of AD than conditioned fear responses.

Disclosures: B. Schuessler: None. **N. Kim:** None. **J. Jang:** None. **J. Kim:** None. **E. Kim:** None.

Poster

P219. Roles of Amyloid Beta in Cognition and Behavior

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P219.03

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

Support: NIH Grant DS044242
NIH Grant U54AG054345

Title: Impaired cognitive function and reduced nicotine intake in male TgF344-AD rats

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Abstract: Background: Alzheimer's disease (AD) is associated with various neuropsychiatric symptoms, including motor disturbance and cognitive dysfunction. Tobacco smoking is a risk factor to the development of AD. Nicotine is the major addictive component of tobacco and has been shown to improve cognitive function. We hypothesize that AD rats would consume more nicotine than control rats due to the cognition-enhancing effects of nicotine serving as a self-medication mechanism, which may exacerbate the negative impact of tobacco smoking on AD. **Method:** Male TgF344-AD rats and wild-type (WT) F344 rats (n=15-18/genotype, starting ~ 6 months) were assessed for basal locomotor activity (LMA) in an open field test followed by spatial learning and memory function in a radial arm maze (RAM) test reinforced by a piece of Froot Loops at the distal end of each arm. Three primary parameters were assayed: (1) entries-to-repeat (ETR) defined as the total number of arm entries before making an error by repeating an arm entry, (2) session time defined as either the maximum of 300 seconds or time during which rats finish entry to all arms, whichever comes first, and (3) Froot Loops consumed. Following the RAM test, rats were trained for operant intravenous self-administration (IVSA) of nicotine (0.03 mg base/kg/infusion). **Results:** AD and WT rats did not differ in ambulatory distance travelled across sessions (p = 0.28). Compared to AD rats, WT rats achieved significantly greater numbers of ETR (p = 0.000, 4.3 vs 2.1/session), completed sessions significantly faster (p = 0.000, 251 vs 290 s/session), and consumed significantly more Froot Loops (p = 0.001, 4.2 vs 1.6/session). Data from IVSA revealed significant effect of session x genotype interaction (p = 0.026), with significantly more infusions in WT rats than AD rats toward the last week of IVSA (p = 0.024, 8.5 vs 1.4 infusions/session). **Conclusions:** Our data indicate that TgF344-AD rats display normal basal LMA and impaired spatial learning and memory function, but didn't consume more nicotine than WT rats. These results suggest the hypothesized cognition-enhancing effects of nicotine may not serve as a self-medication mechanism to increase nicotine intake in AD rats.

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Poster

P219. Roles of Amyloid Beta in Cognition and Behavior

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Program #/Poster #: P219.04

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

Support: NIH Grant R01AG057767
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the Dale and Deborah Smith Center for Alzheimer's Research and trEatment, and the Kenneth Stark Endowment

Title: Oral senolytic treatment improves cognition and metabolism female APP $^{NL-F}$ knock-in Alzheimer's disease mice

Authors: *Y. FANG, D. MEDINA, R. STOCKWELL, S. MCFADDEN, K. QUINN, M. PECK, L. ALEWELT, A. BARTKE, K. HASCUP, E. HASCUP;
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Abstract: Cellular senescence is characterized by an irreversible cell-cycle arrest accompanied by distinctive secretory phenotypes. Senescent cells accumulate in numerous tissues and organs, and contribute to functional alterations associated with aging and neurodegenerative diseases including Alzheimer's disease (AD). A causal link has been shown between the accumulation of senescent cells and cognition-associated neuronal loss. Deletion of senescent cells genetically or pharmacologically with senolytic drugs in the mouse brain led to functional improvements in AD models. However, it is unclear if the stage of disease progression at intervention or sex play a role in treatment response. Here, we examine the effects of early and prolonged senolytic drug treatments in the APP^{NL-F} knock-in mouse model of AD. Male and female APP^{NL-F} knock-in mice were orally administered Fisetin (100 mg/kg BW), Dasatinib (5 mg/kg BW) plus Quercetin (50 mg/kg BW; D+Q), or vehicle control starting at 4 months of age. The treatments were carried out once/month for 8 months. At the end of treatment, all mice underwent glucose tolerance test (GTT) and insulin tolerance test (ITT) to examine glucose metabolism, indirect calorimetry to determine energy metabolism, and the Morris water maze (MWM) to ascertain cognitive abilities. The results revealed that senolytic treatments did not alter glucose metabolism in APP^{NL-F} knock-in mice (GTT and ITT). Senolytic treatment altered the energy metabolism in a sexually dimorphic manner whereby D+Q treatment increased oxygen consumption (VO2) and energy expenditure (EE) in female APP^{NL-F} knock-in mice accompanied with significant reduction of abdominal adipose tissues, while Fisetin decreased respiratory quotient (RQ) in male APP^{NL-F} knock-in mice. Importantly, D+Q treatments in female APP^{NL-F} knock-in mice led to improved long-term memory retrieval (MWM). Together, these data support improved spatial learning and memory in D+Q treated female APP^{NL-F} knock-in mice accompanied with the enhanced peripheral energy metabolism and decreased abdominal adiposity.

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Poster

P219. Roles of Amyloid Beta in Cognition and Behavior

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Program #/Poster #: P219.05

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

Support: NINDS R01 NS110749 (KLZ)
NINDS Predoctoral Fellowship F31 NS115290 (OJG)

Title: Females have it worse: In mouse models of Alzheimer's disease and mixed dementia females are more severely affected by a high fat diet than males

Authors: *O. J. GANNON[1], L. S. ROBISON[2], C. ABI-GHANEM[1], F. M. MANSOUR[1], A. E. SALINERO[1], R. BRAWLEY[3], A. TYAGI[1], K. L. ZULOAGA[1];
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Abstract: Diabetes and prediabetes are major risk factors for all-cause dementia. 1 in 10 people in the United States currently have diabetes- a figure that is on the rise. Prediabetes is even more common, affecting roughly 1 in 3 in the U.S. who are frequently unaware of this diagnosis. There is much to learn about how prediabetes can contribute to dementia decades later and examining this relationship may unveil new targets or emphasize prediabetes as a risk factor. Here we examine the impact of prediabetes on two of the most common types of dementia: Alzheimer’s disease (AD) and mixed dementia (MxD) which is the overlap between AD and vascular pathology. Furthermore, given that there are sex differences in both the prevalence and presentation of dementia types, we ask if prediabetes influences males and females differently. There is evidence to suggest the heightened severity of diabetes as a dementia risk factor for women: women with diabetes have a 19% greater risk of vascular dementia than men with diabetes. We hypothesize that female mice will face a greater degree of pathology in response to prediabetes modeled by a high fat diet and that this will exacerbate AD and vascular pathology in mouse models of AD and MxD. Using male and female B6129SF2/J mice as wild type (WT) controls, we modeled AD using the 3xTg-AD mouse model of Alzheimer’s disease and MxD by performing a unilateral common carotid artery occlusion surgery to produce chronic cerebral hypoperfusion. To model prediabetes, we administered a high fat diet (60% fat). Control mice received a control diet (10% fat). We found that high fat diet led to greater metabolic effects in females (increased weight gain and glucose intolerance) and that this was exacerbated by 3xTg-AD genotype in females only. We measured classical features of AD pathology: astrogliosis and beta-amyloid burden using IHC and tau pathology using western blot. We have found that females had greater levels of hippocampal astrogliosis and cortical beta-amyloid positive cells than males with no effect of diet. However, MxD trended toward increasing amyloid pathology only in high fat fed females ($p=0.06$). In sum, our findings demonstrate increased sensitivity of females to the negative metabolic effects of high fat diet and the pathological effects of AD and MxD.

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Poster

P219. Roles of Amyloid Beta in Cognition and Behavior

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P219.06

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

Support: TCU Science and Engineering Research Center

Title: Effects of unpredictable stress on cognitive function and amyloid-beta in C57BL6/J mice

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Abstract: Alzheimer's disease (AD) is a progressive form of dementia marked by severe cognitive decline and memory loss for which there is no cure. Around 6.2 million people in the U.S. suffer from this devastating neurodegenerative disease, and these numbers continue to increase. For most AD cases, the causes underlying the development of the disease are not well understood. Evidence suggests that lifestyle-related environmental factors may be partially to blame, including significant chronic stress. Unfortunately, over two-thirds of U.S. adults report regularly experiencing stress that negatively impacts their mental and physical health, with unusual increases in stress occurring during the year 2020. Previous research has shown that individuals with mild cognitive impairment (MCI) who underwent chronic stress were more likely to develop AD compared to those with MCI who did not experience chronic stress. Furthermore, amyloid-beta, a protein that aggregates abnormally to create plaques that disrupt neural communication in AD, is elevated following extended activation of the HPA axis. Moreover, inflammatory processes that have been implicated in the initiation and progression of AD, occur in response to increased glucocorticoids in the brain. To further explore these relationships, the present study investigated the interaction between unpredictable stress, inflammation, and AD-like pathology in non-transgenic C57BL/6 mice. Previous work in our lab has shown that mice administered seven consecutive days of LPS, a bacterial mimetic, exhibit cognitive impairment and increased proinflammatory cytokines and amyloid-beta in the brain. In Experiment 1, adult C57BL/6 mice underwent 7 days of unpredictable stress, after which contextual fear learning and extinction learning were assessed. In Experiment 2, adult C57BL/6 mice underwent 21 days of unpredictable stressors and received LPS injections during the final 7 days of the stress paradigm. Following the final day of stress and injections, mice underwent contextual fear conditioning to assess cognitive function, and hippocampal amyloid-beta was quantified. Although, in the current studies, LPS administration had no effect on amyloid-beta or cognition, both 7 days and 21 days of unpredictable stress were associated with learning impairments in contextual fear conditioning. Furthermore, 21 days of chronic unpredictable

stress led to a slight increase in hippocampal amyloid-beta. Given the large percentage of U.S. adults who report experiencing chronic stress and the continued increase in AD prevalence, understanding the link between significant psychological stress and AD is imperative.

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Poster

P219. Roles of Amyloid Beta in Cognition and Behavior

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

Support: NSF Grant 1944247
Alzheimer's Association AARG-2018-59104
Emory ADRC grant P50 AG025688
Emory ADRC grant P30 AG066511

Title: Quantifying effects of amyloid beta reduction with BACE inhibitors in Alzheimer’s mice

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Abstract: Beta-secretase (BACE) is an enzyme that cleaves the amyloid precursor protein (APP). In recent years, BACE inhibiting drugs have become the focus of much research into the development of treatments for Alzheimer’s Disease (AD) with the intent that inhibiting the cleavage of APP will prevent the formation of amyloid-beta plaques and, consequently, cognitive decline. In the past, several BACE1 inhibitors have been developed; however, evidence for positive effects in both animals and humans has been minimal. This study focuses on quantifying the level of BACE and amyloid-beta level reductions necessary to significantly improve cognition in transgenic AD mice as measured via Morris Water Mouse (MWM). In order to observe the effects of BACE inhibitors on the production of amyloid-beta and the cognitive effects it had on mice, a meta-analysis was performed using a database of over 3,000 Alzheimer’s mouse model peer-reviewed articles. Using the keywords “Alzheimer’s disease, amyloid-beta, beta-secretase, and Morris Water Maze”, 23 acceptable peer-reviewed studies were found. Escape latency of Morris Water Maze, both the insoluble and soluble 40/42 amyloid-beta levels, and the BACE levels were extracted from each study and included for analysis. An unsupervised machine learning analysis was performed on the data using principal component analysis followed by k-means clustering method to identify separable clusters based primarily on amyloid-beta 40 and 42 levels. When comparing amyloid-beta 42 to escape latency, clustering showed a 25 percent decrease in amyloid-beta led to a 10 percent decrease in escape latency in AD mice, while a 40 percent decrease in amyloid-beta led to a 20 percent decrease in

escape latency. For reference, wild-type (WT) mice have an escape latency 25 percent less than the AD model. Chi-square statistical analysis confirmed a significant difference in cognition in transgenic AD mice groups based on three different levels of amyloid-beta reduction using BACE inhibitors. Results demonstrate that amyloid-beta needs to be greatly reduced, by about 40 percent, in order to achieve WT levels of cognition. These findings conclude BACE inhibitors could have a significant effect on the cognitive decline of patients with either familial or sporadic AD but require a tremendous amount of amyloid-beta reduction to achieve it. Thus, it is likely that BACE inhibitor treatment in humans would need to start pre-onset by identifying patients with predictive biomarkers well before there are functional symptoms of AD.

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Poster

P219. Roles of Amyloid Beta in Cognition and Behavior

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Program #/Poster #: P219.08

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

Support: NIH Grant R37AG008796
NIH Grant RF1AG017139

Title: Spatial memory deficits are observed across the lifespan of TgF344-AD rats, a transgenic rat model of Alzheimer’s disease

Authors: *M. M. OH[1], C. HICHERI[1], A. S. BUELL[1], C. LIN[1], C. SPAENI[1], N. VILCEK[1], D. NICHOLSON[2], C. GEULA[3], J. F. DISTERHOFT[1];
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Abstract: TgF344-AD is a transgenic rat model of Alzheimer’s disease (AD) that has been designed to express the human amyloid precursor protein harboring the Swedish mutation (*APP_{SW}*) and human presenilin 1 with the αE9 mutation (*PS1αE9*) on the Fischer 344 rat background, which leads to aging-related AD pathology: e.g., increased amyloid plaques, hyperphosphorylated tau and cell loss at old age as compared to young adults (PMID:23575824). The initial study also showed that the middle-aged (15 month old: mo) TgF344-AD rats were impaired in spatial learning on the Barnes dry/land version of the water maze task, as compared to their wild-type littermates (WT). No difference in learning rate was reported between the young adult (6mo) TgF344-AD and WT rats, and only a deficit on the last day of training was reported in the aged (24mo) TgF344-AD rats as compared to the aged WT rats. A number of other reports has tested the learning ability of young adult and middle-aged TgF344-AD rats on

the spatial water maze task and have found altered search strategies without deficits on the location of the hidden-platform, but impairments on reversal learning. Given the potential impact of age-related AD pathology in TgF344-AD rats on learning, we examined their performance on the spatial water maze task in a cross-sectional study: in young adult (6-8mo), middle-aged (12-14mo) and aged (18-22mo) rats. We did not observe sex (male, M; female, F) differences on the water maze task, similar to previous reports using TgF344-AD rats. Compared to young adult WT rats (9F, 14M), we found that 13/28 (46%) young adult (11F, 17M), 10/33 (30%) middle-aged (11F, 22M) and 61/79 (77%) aged (45F, 34M) TgF344-AD rats were impaired on the water maze task, while 4/8 (50%) middle-aged (2F, 6M) and 45/77 (58%) aged (44F, 33M) WT rats were impaired. We have also trained a subset of aged TgF344-AD and WT rats on the trace eyeblink conditioning task and found that 12/40 (30%) WT and 49/76 (64%) TgF344-AD rats were unable to learn this task. Additional trace eyeblink conditioning will be conducted using young adult and middle-aged rats. These are the first comprehensive cross-sectional experiments that show increasing proportion of transgenic rat population that display learning deficits in young adult rats that increase as they age. We hypothesize that the aging-related learning deficits are correlated with increasing AD pathology in these TgF344-AD rats. Immunohistochemical experiments examining amyloid plaques, hyperphosphorylated tau, and cell loss are ongoing to test our hypothesis.

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Poster

P247. Biomarkers in Neurodegenerative Diseases VI

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Program #/Poster #: P247.01

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

Support: NIH Grant 5P20GM109025-05

Title: Inflammatory Biomarkers in Progressive Alzheimer's Disease

Authors: *K. CALVIN[1], L. CREW[1], A. PLATT[1], E. FLORES[1], R. A. WIRT[2], J. M. HYMAN[3]; [1]Univ. of Nevada, Las Vegas, Las Vegas, NV; [3]Psychology, [2]Univ. of Nevada Las Vegas, Las Vegas, NV

Abstract: One of the core features of Alzheimer’s disease (AD) is neuroinflammation, which is driven by the chronic activation of neuroimmune defense cells. Epidemiology and biological research have shown a strong link between neuroinflammation and cognitive decline. Currently there is little known about biological markers for cellular and molecular alterations of inflammation that occur in early disease stages of AD. The present study is a preliminary

glimpse into potential neuroinflammatory biomarkers for early detection that may lead to clinical intervention.
This data was collected from participants at the Center for Neurodegeneration and Translational Neuroscience (CNTN) who are enrolled in a longitudinal, natural history study of neurodegeneration and cognitive function. We reviewed correlations between blood levels of several biomarkers of neuroinflammation; including interleukin-4 (IL-4), interleukin-9 (IL-9), interleukin-15 (IL-15) lymphotoxin-alpha (LT-α), macrophage migration inhibitory factor (MIF), and Chemokine (C-C motif) ligands 4 (CCL4), with amyloid plaque positivity as determined by PET scan. Biomarker levels were assessed via Luminex multiplex assay in participants' first year of data collection. We also reviewed correlations between these biomarkers and a Polygenic Risk Score (PRS) derived from a number of AD risk alleles. Lastly, we determined if there was any variation between participants whose diagnosis remained stable and those whose diagnoses progressed. Participants in this analysis included cognitively normal controls (CNC) as well as those with diagnoses of Mild Cognitive Impairment (MCI), and both mild and moderate AD.Using a one-way ANOVA, we found a significant increase in biomarkers IL-4, IL-9, IL-15, LT-α, MIF, and CCL4 in participants with progressive cognitive impairment diagnoses. Interestingly, when correlated with PRS and amyloid plaque positivity as determined by PET scan, we found a positive correlation in biomarkers IL-9, LT-α, and CCL4.
More analysis is needed; however, given that neuroinflammation has been shown to indicate fast progression of cognitive impairments in AD patients, these preliminary results may be a glimpse into the early detection of AD and intervention. LT-α, IL-9 and CCL4 are important immune regulators, and we found correlations with each of these biomarkers and PRS and amyloid status, as well as an increase in levels in participants with progressive cognitive decline diagnoses. Biomarker levels were tested during participants' first year of study participation, indicating that these correlations could be potential early markers that appear prior to disease progression.

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Poster

P247. Biomarkers in Neurodegenerative Diseases VI

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Program #/Poster #: P247.02

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

Title: Neuroimaging biomarkers in aging: testing the independence of white matter hyperintensity volume, dopamine synthesis, and Alzheimer's disease-related pathology

Authors: *J. EPSTEIN[1], C. J. CIAMPA[2], J. H. PARENT[2], V. R. TENANT[4], V. D. SHAH[4], T. M. HARRISON[4], W. J. JAGUST[5], A. S. BERRY[3];

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Abstract: Identifying neuroimaging biomarkers associated with cognitive decline, as well as identifying potential interactions between biomarkers, is critical for advancing treatment of the growing aging population. Previous research from Rieckmann and colleagues identified associations between white matter hyperintensity (WMH) volume and multiple dopamine PET measures (transporter availability, D1 receptor availability), but found no relationship between WMH volume and Alzheimer's disease (AD)-related β-amyloid pathology in cognitively normal older adults (Rieckmann et al., 2016). We replicated these analyses in a sample of cognitively normal older adults (n=47, average age=77) using the dopamine PET tracer 6-[^{18}F]fluoro-L-*m*-tyrosine ([^{18}F]FMT) to measure dopamine synthesis capacity, [^{11}C]Pittsburgh compound B ([^{11}C]PiB) to measure β-amyloid, and 3T fluid-attenuated recovery inversion (FLAIR) magnetic resonance imaging to measure WMH volume. Further, we probed relationships with AD-related tau pathology using [^{18}F]Flortaucipir PET, and cognition using a composite measure of processing speed (Trails A Test, Stroop neutral condition, and the Digit Symbol test). We did not find a relationship between WMH volume and the [^{18}F]FMT net tracer influx (Ki) measure of striatal dopamine synthesis capacity (beta = .063, p= .714). However, for a model including entorhinal cortex [^{18}F]Flortaucipir standardized uptake value ratio (SUVR), we observed a significant interaction between WMH volume and EC [^{18}F]Flortaucipir SUVR in predicting striatal [^{18}F]FMT Ki ($beta_{interaction\ term}$ = .517, p= .048). Greater WMH volume was associated with higher striatal [^{18}F]FMT Ki for those participants with greater tau burden. The positive association between [^{18}F]FMT Ki and WMH is consistent with our previous findings suggesting elevated dopamine synthesis is a compensatory mechanism in aging. We found no overall association between WMH and EC [^{18}F]Flortaucipir SUVR (r=-.047, p=.789) nor WMH and [^{11}C]PiB status (r=-.046, p=.789). Consistent with reports linking WMH and processing speed performance, we found greater WMH volume was associated with reduced processing speed (beta=-.421, p=.019). There was an additive effect of EC [^{18}F]Flortaucipir SUVR (WMH beta=-.436, p=.015; tau beta= -.343, p=.050) revealing white matter integrity and tau burden as critical measures of interest in understanding the neurobiological mechanisms underlying individual differences processing speed in cognitively normal older adults.

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Poster

P247. Biomarkers in Neurodegenerative Diseases VI

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Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P247.03

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

Title: Quantitation of neuronal proteins Nf-L, t-Tau, pTau181, and inflammatory cytokines IL-6, IL-10, TNFa in Alzheimer's disease plasma

Authors: *A. CHENNA, J. W. WINSLOW, C. J. PETROPOULOS;
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Abstract: There are more than 6 million Americans who have Alzheimer's disease (AD), currently ranked as the sixth leading cause of death in the U.S. Changes in blood levels of neuronal proteins (NF-L, t-Tau, p-Tau181) and inflammatory cytokines (IL-6, IL-10, TNFα) are associated with brain cellular damage, and are actively being explored as as possible prognostic/diagnostic biomarkers of AD. The low blood concentrations of several of these proteins require application of a sensitive and reproducible assay technology, therefore, single molecular array (Simoa) immunoassays were used to measure protein levels. In this study, we have characterized the protein markers NF-L, t-Tau, pTau181, IL-6, IL-10, and TNFα in human AD plasma (n=72) and healthy human control plasma (n=64). AD plasma sample groups consisted of mild (n=8, MMSE > 23-30), moderate (n=24, MMSE 16-22) and severe (n=40, MMSE <16) cognitively impaired AD. Concentrations (pg/mL) were determined with machine dilutions 4x for all plasma samples. Data analysis measuring differences of each of the plasma proteins within control and AD patient subgroups, was performed together (Krustal-Wallis) or pairwise (Mann-Whitney). For NF-L, t-Tau, p-Tau181, IL-6, IL-10, and TNFα, there is a significant increase in median levels of all proteins in the combined AD group (n= 72; $p < 0.0001$), relative to controls (n= 64). The levels of all biomarkers are elevated in the moderate and severe AD patient subgroups ($p < 0.0001$). Similar results were observed in a comparison of moderate AD samples (n=24; ave age = 75.6) with an age-matched subset of control samples (n= 30; ave age = 72.1) ($p \leq 0.0004$). There was no significant difference between all 6 protein levels in plasma samples from mild AD patients compared to control samples, however, the sample size is small (n= 8). The plasma neurodegenerative biomarkers NF-L, p-Tau181, and t-Tau were significantly correlated to each other in the AD group (Spearman $r = 0.5\text{-}0.7$, $p < 0.0001$) as were the three cytokines (IL-6, IL-10, TNFα: $r = 0.5\text{-}0.7$; $p<0.0001$). Cross-correlation between neurodegenerative proteins and cytokines indicated the strongest correlation between NF-L and t-Tau and the three cytokines ($r = 0.5\text{-}0.66$, $p<0.0001$), whereas correlations between p-Tau181 and cytokines were either weaker (IL-10, TNFa $r = 0.37\text{-}0.41$; $p = 0.0015$; 0.0004) or not significant (p-Tau181 vs IL-6; $r = 0.155$; $p = 0.197$). In summary, moderate and severe AD plasma samples were significantly elevated in levels of NF-L, t-Tau, p-Tau181, IL-6, IL-10, and TNFα. A combination of plasma neuronal neurodegenerative and inflammatory cytokine biomarkers may effectively identify AD patients.

Disclosures: A. Chenna: A. Employment/Salary (full or part-time):; Monogram Biosciences Inc/LabCorp. **J.W. Winslow:** A. Employment/Salary (full or part-time):; Monogram Biosciences Inc/LabCorp. **C.J. Petropoulos:** A. Employment/Salary (full or part-time):; Monogram Biosciences Inc/LabCorp.

Poster

P247. Biomarkers in Neurodegenerative Diseases VI

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P247.04

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

Support: NIH Grant R21NS118079
R01 NS082338

Title: Perturbed cerebral glucose uptake and glymphatic system in a mouse model of Huntington's Disease

Authors: *H. LIU[1], L. CHEN[2], C. ZHANG[3], L. CHENG[4], J.-L. DONG[4], J. HUA[2], C. A. ROSS[6], P. ZIJL[2], J. XU[2], W. DUAN[5];
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Abstract: The clinical diagnosis of Huntington's disease (HD) is currently based on the presence of movement disorders. However, subtle-to-prominent brain functional changes can precede by many years the motor-based onset of HD. Given the availability of predictive genetic testing, it is possible to identify HD subjects before motor symptoms are present. This provides a unique opportunity to identify potential early biomarkers to be used for evaluating the disease-modifying therapies. Glucose Chemical Exchange Saturation Transfer (glucoCEST) MRI is a newly developed technique that can detect unlabeled glucose at physiologically relevant concentrations using proton-only MRI scanners. Our recently improved MRI sequence, on-resonance variable delay multiple pulse (onVDMP), allows the detection of low concentration glucose through the chemical exchange of protons between hydroxyl groups and has the capacity to measure glucose clearance from the cerebrospinal fluid (CSF). In this study, we applied onVDMP MRI to measure glucose uptake in the brain and its clearance from the CSF after intravenous injection of D-glucose in the zQ175 HD mouse model. By comparing the DGE curves and maps, the glucose uptake in the striatum of HD mice (4.4 ± 1.35 %) was significantly reduced comparede to the age and gender-matched control mice (7.16 ± 1.36 %, p =0.012), suggesting the presence of an impaired glucose transporter at the blood-brain and blood-CSF barriers of zQ175 mice. . Interestingly, a significantly slower glucose clearance from CSF was found in the CSF of zQ175 HD mice compared to the age and gender-matched controls by examining the CSF DGE signal at 30 min with respect to the signal at 10 min (DGE[30min - 10min]=2.96± 1.7% for HD vs -1.7± 1.7% for WT, $p<0.05$)., suggesting the presence of an impaired glucose transporter at the blood-brain and blood-CSF barriers of zQ175 mice. Further mechanistic molecular study confirmed indicated decreased levels of astrocyte water channel aquaporin-4 (AQP4, ~ 40% reduction in HD mice) in the striatum of zQ175 mice. These results suggest imply that a dysfunctional glymphatic system are evident in the zQ175 HD mouse

modelmice. and onVDMP MRI measures have the potential to be further developed as valuable biomarkers to monitor disease progressionfor HD and evaluate potential treatment for HD.

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Poster

P247. Biomarkers in Neurodegenerative Diseases VI

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P247.05

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

Title: Utilizing RabMAb®Tau antibodies for targeting different PTM sites aiding the identification of AD subjects

Authors: *M. DROESCHER[1], I. WOLLENBERG[2], J. BERNHARD[1], H. WIELAND[1], A. KARPATI[2], S. BARGHORN[1];
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Abstract: Background The current clinical screening methodology for Alzheimer's disease is resource-intensive and results in a high screening failure rate identifying genuine AD-subjects. Although the acquisition is considered an invasive procedure, for cerebrospinal fluid several well validated technologies are available. However, the desired analysis of non-invasively acquired blood samples for informative biomarkers, still poses a great challenge. **Objectives**Cross-characterization of multiple pTau biomarker assays to identify potential to aid robust identification of patients with underlying AD pathology aiming not only for resolution of CSF but also of plasma matrices.**Methods**Novel assays were developed using rabbit monoclonal capture antibodies with high binding affinities. Next to a discovery cohort encompassing soluble brain extracts, CSF and plasma from different donors serving the direct comparison, a Phase 0 AD cohort with matching CSF and plasma samples was used to compare the performance of pTau181, pTau217 and pTau231.**Results**In the CSF matrix, the pTau assay data show a very strong positive correlation with each other (Spearman R's of >0.90). Interestingly, also the very C-terminal pTau396 data correlated well with the described modifications of pTau181 and pTau231. However, in the plasma matrix strong correlations were observed only for some distinct pTau pairings. Notably, the modifications in the MTBR and C-terminal region, pTau262/263 and pTau396, showed a
reduced correlation with other pTau assays. Overall, we describe 8 PTM site-specific assays resolving the CSF and plasma matrix. While in CSF all assays achieve the discrimination of AD to Control subjects, in plasma only 5 assays accomplished this. In a more powered cohort with matching CSF and plasma, pTau231 was identified as the most robust assay. pTau231 not only

achieved significant differentiation of the AD and Control groups (both $p<0.001$). A remarkable performance of AUC = 0.87 was also reached for predicting Amyloid-PET positivity and differentiating MCI-Aß$^{+}$ and MCI-Aß$^{-}$ groups ($p=0.002$).**Conclusion**Blood-based pTau biomarkers have great potential to aid the diagnosis of patients with underlying AD pathology, to simplify both clinical and pre-clinical study designs, reduce patient burden, as well as enabling the continuous monitoring of the effect of potential therapeutic drug candidates.**Disclosures**MD, JB, HW, and SB are employees of AbbVie. IW, MF, and AK are employees of Abcam. The design, study conduct, and financial support for this research were provided by AbbVie. AbbVie and Abcam participated in the interpretation of data, review, and approval of the publication.

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Poster

P247. Biomarkers in Neurodegenerative Diseases VI

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Program #/Poster #: P247.06

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

Support: NIH Grant R21AG051987

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Title: Higher tau deposition in the dorsal attention network is associated with visual attention deficits in posterior cortical atrophy

Authors: *Y. KATSUMI, D. PUTCHA, R. ECKBO, B. WONG, M. QUIMBY, S. MCGINNIS, A. TOUROUTOGLOU, B. C. DICKERSON;
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Abstract: Localization of tau pathology, as measured with 18F-flortaucipir (FTP) PET imaging, is highly relevant for characterizing clinical phenotypes of Alzheimer's disease, including posterior cortical atrophy (PCA). The spatial topography of covariance in tau accumulation across Alzheimer's disease patients resembles the topography of intrinsic functional connectivity in healthy young adults, suggesting that tau pathology spreads though functional networks in the cerebral cortex. The present study investigated the degree to which the cortical distribution of elevated FTP uptake varies across individual patients with PCA, with the goal of identifying functional networks of heightened vulnerability to tau pathology in this population. Seventeen patients with PCA and 22 amyloid negative older healthy control subjects underwent MRI and FTP PET scans. Individual FTP standardized uptake value ratio maps were computed and projected to template cortical surface space, which were converted to W-score maps to identify cortical areas with elevated FTP uptake. Percent overlap maps were created based on the binarized individual W-score maps. A parcellation of the cerebral cortex into seven intrinsic functional networks was used to characterize the spatial topography of elevated FTP uptake. At the overall group level, PCA patients showed prominent FTP uptake in widespread areas of the cerebral cortex including bilateral frontal eye fields, superior parietal lobule, and area MT, which are key nodes of the dorsal attention network (DAN). An examination of the consistency of elevated FTP uptake across each individual PCA patient showed that the visual network and DAN were most consistently affected, with 100% of the patients showing elevated uptake in at least some parts of the two networks. Impaired visual attention was associated with elevated FTP uptake specifically in the anterior DAN (bilateral frontal eye fields). The ratio of elevated FTP uptake between the anterior vs. posterior DAN nodes was the strongest correlate of visual attentional impairment, suggesting that PCA patients who had more advanced tau spread from posterior to anterior nodes within the DAN showed greater impairment in visual cognition. These results support the hypothesis that tau pathology in PCA universally affects the key nodes of the DAN consistent with the visual attention deficits commonly observed in PCA, including oculomotor apraxia (impaired voluntary control of eye movements). Although cross-sectional, these data provide strong support for the hypothesis that tau deposition in PCA begins posteriorly and that visual cognition declines as it propagates within the DAN.

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Poster

P247. Biomarkers in Neurodegenerative Diseases VI

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Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P247.07

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

Support: SIU New Collaboration Grant

Title: Chronic circadian disruption in an AβPP/PS1 and $APP^{NL\text{-}F/NL\text{-}F}$ model of Alzheimer's disease

Authors: *J. BRITZ[1], E. R. HASCUP[2], K. N. HASCUP[2], E. OJO[2], S. TISCHKAU[2];
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Abstract: The circadian system uses environmental cues to coordinate the plethora of physiological functions that occur with diurnal rhythmicity, with light being one of the strongest regulators of the circadian system. The suprachiasmatic nucleus (SCN) is positioned at the top of the circadian hierarchy, receiving photic input from the retina and using neuronal, humoral and endocrine signals to synchronize oscillatory patterns across virtually all organ systems. Though the circadian system is plastic to deviations from a normal light/dark (LD) cycle, there are biological limits as to the rate and degree to which the SCN can adapt to these deviations. Recent evidence suggests that circadian disruptions may precede noticeable cognitive deficits associated with AD. Our study aims to determine whether inducible circadian disruption via exposure to our social jetlag protocol, taking advantage of manipulating light exposure to disrupt the circadian system, can exacerbate the rate and severity of AD pathology in both the AβPP/PS1 and $APP^{NL\text{-}F/NL\text{-}F}$ mouse models of AD. WT; C57BL/6J controls, AβPP/PS1 and $APP^{NL\text{-}F/NL\text{-}F}$ AD models were entrained to a control light/dark cycle starting at 6 weeks of age. Following 2-weeks of entrainment, half of the animals were maintained on the control schedule, and half were exposed to our circadian disruption paradigm, involving an 8-hour phase advance on day 5 and an 8-hour phase delay on day 7 of repeated 7-day sessions up until 6 or 12-months. One month prior to the endpoint, activity measures and GTT were performed, following which the animals were all entrained to a normal 12:12 LD schedule for 3-5 weeks. Finally, animals underwent an 8-day Morris Water Maze (MWM) to assess effects of chronic disruption on AD-related cognitive decline. Animals were sacrificed and tissues collected at Zeitgeber time (ZT) 12. In all genotypes, female animals showed a higher degree of re-entrainment to the phase advancement of the lighting schedule going into the subjective weekend (day 6-7). Arginine vasopressin (AVP) and vasoactive intestinal peptide (VIP) expression in the SCN were significantly dampened as a result of chronic disruption in 12-month male AD mice. Amyloid plaque analysis indicated a severely worsened pathological phenotype in AβPP/PS1 mice as compared to age and sex-matched $APP^{NL\text{-}F/NL\text{-}F}$ mice. MWM data provides evidence for impaired spatial learning in both AD models that is significantly worsened by chronic jetlag exposure. Taken together, the data suggests that chronic exposure to the social jet lag schedule disrupts rhythmic behavior, and spatial learning significantly in both AD animal models.

Disclosures: J. Britz: None. **E.R. Hascup:** None. **K.N. Hascup:** None. **E. Ojo:** None. **S. Tischkau:** None.

Poster

P248. Parkinson's Disease: Cellular and Network Dysfunction

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P248.01

Topic: C.03. Parkinson’s Disease

Title: Disrupted projection signaling in sleep disorders and parkinsonism

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Abstract: Healthy humans switch seamlessly between activity states, wake up and fall asleep with regularity, and cycle through sleep stages necessary for restored homeostasis and memory consolidation each night. This study examined whether such smooth behavioral transitions are accompanied by low noise transitions between stable neural states within the brain. We found that electrical brain activity measured in pathological conditions of 1) impaired human sleep and 2) non-human primate parkinsonism displays significantly increased phase disruption compared to healthy controls. Our analyses used a new set of measures we recently developed to detect and quantify disruptions in EEG signals across a broad range of frequencies [1], here referred to as “phase discontinuity” measures. In humans reporting impaired sleep, we found that phase discontinuity decreased with increasingly deep sleep, and that greater and more variable phase discontinuities were associated with increased drowsiness, impaired deep sleep, and shorter REM sleep. A 10 sec phase discontinuity summary measure (the *phase jump indicator*) closely tracked the annotated sleep stages and enabled discrimination between short (< 10 min) and longer REM periods. Increased phase discontinuity only occurred in sleep stages that showed impairment. A measure of total phase discontinuity correlated inversely with broadband EEG power, suggesting that reduced spurious signaling may facilitate increased synchronization. In older patients the power vs. phase discontinuity correlation shifted to positive during drowsiness, suggesting potential deficits in inhibition. These results provide evidence that phase discontinuity measures extend current capabilities for assessing sleep and may yield new insights into pathological brain states. In monkeys with Parkinsonism, we found that phase discontinuities are also increased in cortical and subthalamic LFPs. We show via modeling how circuit changes that occur during Parkinson’s disease may lead to increased disruptions in cortico-basal ganglia signaling that can be quantified with phase discontinuity measures.
1) https://www.biorxiv.org/content/10.1101/696658v2

Disclosures: T. Sanders: None.

Poster

P248. Parkinson's Disease: Cellular and Network Dysfunction

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Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

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Topic: C.03. Parkinson's Disease

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Title: Differential engagement of the pallidum during wake and REM sleep movements in Parkinson's disease

Authors: S. ACOSTA LENIS[1], A. VERMA[1], J. E. AMAN[2], D. ESCOBAR SANABRIA[3], J. WANG[3], A. PEARSON[3], M. HILL[3], R. PATRIAT[4], L. E. SCHROCK[3], S. E. COOPER[6], M. C. PARK[5], N. HAREL[8], M. J. HOWELL[1], C. D. MACKINNON[3], J. L. VITEK[7], *L. JOHNSON[1]; [2]Dept. of Neurol., [3]Neurol., [1]Univ. of Minnesota, Minneapolis, MN; [4]CMRR / Radiology, Univ. of Minnesota, Woodbury, MN; [5]Neurosurg., Univ. of Minnesota, Minneapolis, MN; [6]Neurol., [7]Dept. of Neurol., Univ. of Minnesota Dept. of Neurol., Minneapolis, MN; [8]Radiology, Univ. of Minnesota Syst., Minneapolis, MN

Abstract: Rapid eye movement (REM) sleep behavior disorder (RBD) is often co-expressed with Parkinson's disease (PD) and in many cases occurs before the overt motor signs of PD. This has led to increased interest in the mechanisms underlying movements during REM sleep. In this study we sought to increase our understanding of basal ganglia involvement in REM sleep movements and whether it is engaged differently than wakeful movements. Local field potentials from directional deep brain stimulation (DBS) leads in the globus-pallidus internus (GPi) and scalp EEG polysomnography were recorded in four PD patients (one identified with RBD) and movement-related changes in oscillatory activity in wake and REM states were characterized. During wakeful movements, all patients showed desynchronization of beta (13-35Hz) and synchronization of high-frequency oscillations (150-350Hz). Conversely, during REM sleep movements, all patients exhibited beta synchronization in addition to synchronization of high-frequency oscillations. Our findings suggest that REM sleep movements irrespective of RBD are likely not mediated by the same motor circuit as during wakefulness.

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Poster

P248. Parkinson's Disease: Cellular and Network Dysfunction

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Topic: C.03. Parkinson's Disease

Support: Spanish Ministry of Science and Innovation (PID2019-111693RB-I00)
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SAF2016-77758-R AEI/FEDER

Title: Non-motor symptoms and altered Dorsal Raphe oscillatory activity in a mice model of Parkinson´s Disease

Authors: S. VEGAS-SUAREZ[1], A. SANZ-MAGRO[1], N. GRANADO[1], L. UGEDO[2], *R. MORATALLA[3];
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Abstract: The dorsal raphe nucleus (DRN) is a brainstem nucleus that contains a heterogeneous population of neurons, among which serotonergic neurons predominate. The DRN is implicated in several cognitive, motor and affective functions and its alteration has been linked with the onset of non-motor symptoms of Parkinson's disease, like anxiety and depression. In this work, we used the aphakia mice, a genetic model that mimicked mild-mid stages of Parkinson's disease to link the non-motor symptoms with DRN functional alterations. To address this objective, we analyzed electrical properties through extracellular single-unit recordings in the DRN and oscillatory activity between DRN and motor cortex under urethane-anaesthesia, accompanied by complete behavioural and anatomical characterization. The results suggest that in the aphakia mice, the reduction of dopamine DRN neurons alters serotonin DRN neurons activity, which presented abnormal electrophysiological properties and diminished oscillatory activity together with an enhanced synchronization with the motor cortex. These alterations are concomitant with the presence of anxiety and depressive sings in the aphakia mice. Altogether, these findings suggest that in the aphakia mice, the DRN electrical properties are compromised by the dopaminergic decline and establish the aphakia mice as an appropriate model for the non-motor symptoms in moderate Parkinson's disease.

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Poster

P248. Parkinson's Disease: Cellular and Network Dysfunction

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P248.04

Topic: C.03. Parkinson’s Disease

Title: Characterizing Cellular Activation following L-DOPA Treatment in a Hemiparkinsonian LacZ Rat Model

Authors: M. COYLE, A. CENTNER, N. LIPARI, S. COHEN, E. WHEELIS, T. TOWNER, C. BISHOP;
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Abstract: Parkinson’s disease (PD) is the 2nd most prevalent neurodegenerative disorder in the world, characterized by dopamine (DA) depletion of the nigrostriatal pathway. Although bradykinesia, rigidity, and tremor are considered cardinal motor symptoms, numerous non-motor symptoms frequently precede PD diagnosis such as sleep disturbance, autonomic dysfunction, gastrointestinal issues, depression, and anxiety. While DA replacement therapy (DART) using l-3,4-dihydroxyphenylalanine (L-DOPA) ameliorates motor symptoms, chronic use induces neuroplastic changes affecting DA, serotonin, and glutamate neurotransmission. Consequentially, DART can exacerbate existing PD-associated psychosis (PDAP) symptoms including hallucination, delusion, and affective disorder while also precipitating the development of novel motor and non-motor side effects such as L-DOPA-induced dyskinesia (LID), impulse control disorders, orthostatic hypotension, and de novo psychosis. Understanding the neural underpinning of these changes remains a paramount goal for the field. For this investigation, adult male LacZ Sprague Dawley rats were rendered hemiparkinsonian with an infusion of 6-hydroxydopamine into the left medial forebrain bundle and vehicle infusion into the right. While all rats produce c-fos in active neurons, the LacZ rat genome features an insertion of β-galactosidase (LacZ) after c-fos, resulting in the production of LacZ in all active neurons. As this enzyme is present in all active neurons and cleaves X-gal into an insoluble blue product, neuronal activity can be characterized post-mortem. Following recovery, animals received 2 weeks of daily injections of either L-DOPA (6 mg/kg; s.c.) or vehicle during which all animals underwent the abnormal involuntary movements (AIMs) test. After a brief washout period, animals were administered either L-DOPA or vehicle to investigate the effects of lesion, and acute or chronic L-DOPA treatment. On the last day of the experiment, AIMs severity was monitored for 60 minutes prior to transcardial perfusion. Brains were prepared and X-Gal staining was performed on 40µm tissue slices to quantify active neurons at the time of perfusion

in a region- and sub-region-specific manner across motor and non-motor areas to identify areas demonstrating ectopic activity relative to the sham-lesioned hemisphere . Such findings will illuminate the influences of lesion and treatment and provide clues to the PD features that emerge throughout disease progression.

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Poster

P248. Parkinson's Disease: Cellular and Network Dysfunction

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Topic: C.03. Parkinson’s Disease

Support: NIH Grant F31NS124269

Title: Phosphodiesterase 10A regulation of dopamine signals in striatal neurons.

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[1]Emory Univ., Decatur, GA; [2]Neurosci., [4]Neurol., [3]Emory Univ., Atlanta, GA

Abstract: BACKGROUND: L-Dopa-induced dyskinesias are abnormal involuntary movements that commonly occur with long-term L-Dopa therapy, the primary treatment for Parkinson’s disease (PD). Previous work has revealed decreased striatal expression levels of the cyclic nucleotides, cAMP and/or cGMP, second messengers involved in dopaminergic signaling cascades of striatal projection neurons (SPNs). Cyclic nucleotide levels are regulated by phosphodiesterases (PDEs), and inhibition of these PDEs may help regulate DA signaling in SPNs. PDE10A is highly expressed in the striatum and has substrate affinity for both cAMP and cGMP. To understand the impact of PDE10A function on striatal SPN responses to DA, we assessed the effect of PDE10A inhibition directly on the SPN activity in the non-human primate (NHP) model of PD, starting with the early phases of parkinsonism. METHODS: An MPTP-treated macaque with mild parkinsonism was used for striatal single-cell recordings with local microinjection of a selective PDE10A inhibitor (PDE10A-I). Our approach was to analyze firing changes of SPNs with continuous recordings from the OFF state, to 1 min and 5 min after local PDE-I injection, to 20 min following a systemic L-Dopa administration (ON state) and to the point of peak-dose dyskinesias (whether or not present). We also analyzed motor behavior using standard measures for NHP. RESULTS: In this mildly parkinsonian animal, the activity of SPNs consistently displayed low firing frequencies. In D1R-like SPNs, we observed an increase in firing rate following local injection of PDE10A-I and a synergistic effect of L-Dopa (ON state) sustaining the frequency increase. In D2R-like SPNs, we did not observe a significant change in

firing rate after local administration of PDE10A-I but did see a significant decrease in firing during the ON state compared to the OFF state. We also analyzed the impact of systemic coadministration of L-Dopa (2 doses: optimal and suboptimal) and an escalating dose of PDE10A-I (0-7.5 mg/kg s.c.) on motor outcomes. DISCUSSION: Our previous work has demonstrated that systemic administration of PDE10A-I decreases dyskinesias. Our current data showed coherent and discrete synergistic and antagonistic effects of the PDE10A-I on D1R-like and D2R-like SPNs, respectively, as predicted by the model. These results suggest that more advanced dopamine loss in animals with more severe parkinsonism could increase PDE10A-I effects, particularly antagonizing dopamine signals in indirect SPNs, and this mechanism may explain the antidyskinetic effects of PDE10A inhibitors that we have previously observed.

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Poster

P248. Parkinson's Disease: Cellular and Network Dysfunction

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P248.06

Topic: C.03. Parkinson’s Disease

Support: Instituto de Salud Carlos III. NEUROMETAB
Chair in Neuroscience UAM-Fundación Tatiana Pérez de Guzmán el Bueno (C.C.)

Title: Stereological analysis of striatal projection neurons and interneurons in control and MPTP-treated non-human primate

Authors: *N. LÓPEZ GONZÁLEZ DEL REY[1,2,3], I. TRIGO-DAMAS[1,2], J. A. OBESO[1,2], C. CAVADA[4], J. BLESA[1,2];
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Abstract: The striatum is the largest nucleus of the basal ganglia. Dopamine depletion of the striatum is the crux pathophysiological feature of Parkinson’s disease (PD). The striatum is mainly composed of projection neurons, the medium spiny neurons, which are GABAergic and express the dopamine- and cyclic-AMP-regulated phosphoprotein DARPP-32. The striatum also contains interneurons which modulate and control the striatal output. Most interneurons are GABAergic; various types are identified on the basis of their protein phenotypes: parvalbumin

(Pv+), calretinin (Cr+), neuropeptide Y, somatostatin, nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH+), and tyrosine hydroxylase (TH+). Another non-GABAergic type are the cholinergic interneurons which express the enzyme choline acetyltransferase (ChAT+). These are thought to play an important role in normal physiological responses (i.e. learning, reward, etc.) and basal ganglia disorders. We used unbiased stereological methods to estimate the volume density of every neuron population in the whole extension of the striatum of control monkeys and monkeys treated with MPTP to produce striatal dopamine depletion. The treated monkeys were classified in symptomatic and non-symptomatic groups. All the analyses on the various neuron populations were performed within the same subjects using similar processing conditions and analysis parameters, thus allowing for reliable data comparisons. The projection neurons (DARPP-32+) were the most abundant neuron type in the striatum: they account for ~86% of the total striatal population. The interneurons made the remaining 14%. Among the interneurons, Cr+ neurons were the most abundant type (57.09%; 7.9% of the total striatal neurons), followed by Pv+ neurons (18.24%; 2.6% of the total), NADPH+ interneurons (13.09%; 1.8% of the total), and ChAT+ interneurons (11.04%; 1.5% of the total). The least abundant interneuron type was that expressing TH (0.54%; 0.1% of the total). No significant changes in volume densities occur in any striatal population following DA depletion, except for the TH+ interneurons, which increased in the non-symptomatic subjects and even more in the symptomatic monkeys. The data obtained are relevant for translational studies targeting specific neuron populations of the striatum. Dopaminergic denervation, even when severe, does not cause neuronal loss in any type of striatal neuron. Such resilience has potential pathophysiological implications.

Disclosures: N. López González del Rey: None. **I. Trigo-Damas:** None. **J.A. Obeso:** None. **C. Cavada:** None. **J. Blesa:** None.

Poster

P248. Parkinson's Disease: Cellular and Network Dysfunction

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Program #/Poster #: P248.07

Topic: C.03. Parkinson’s Disease

Support: NIH Grant NS092730
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Abbott (equipment and materials)
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Title: Functional connectivity in a cortico-subthalamic network for a progressive Parkinsonian non-human primate model

Authors: *C. TOTH[1], J. C. BORE[1], B. CAMPBELL[1,4], H. CHO[1], F. PUCCI[2,3], O. HOGUE[2], R. GOPALAKRISHNAN[2], A. G. MACHADO[1,2,3], K. B. BAKER[1,2];
[1]Dept. of Neurosciences, [2]Ctr. for Neurolog. Restoration, [3]Dept. of Neurosurg., Cleveland Clin., Cleveland, OH; [4]Dept. of Biomed. Engin., Case Western Reserve Univ., Cleveland, OH

Abstract: Parkinson's Disease is a degenerative neurological disease that manifests with cardinal motor signs including bradykinesia and tremor. Although beta-band hypersynchrony in the cortico-basal ganglia network is thought to play a role in disease manifestation, resulting effects on network connectivity are unclear. We examined local field potentials from a non-human primate across the naïve, mild, and moderate disease states (model was asymmetric, left-hemispheric dominant) and probed cortico-cortical and cortico-subthalamic connectivity using Granger causality, a measure of directed effective connectivity, and a subsequent graph-theoretic analysis. Our network included the left subthalamic nucleus (L-STN), bilateral primary motor cortices (L-M1, R-M1) and bilateral premotor cortices (L-PMC, R-PMC). Results showed two distinct Granger causality peaks (Peak A at 5-20 Hz, Peak B at 25-45 Hz). Increases in Peak B connectivity and decreases in Peak A connectivity were associated with disease. Induction of mild disease was associated with several changes in connectivity: 1) the cortico-subthalamic connectivity from the L-PMC to L-STN decreased while the reciprocal L-STN to L-PMC increased, 2) both L-M1 to L-PMC and R-M1 to R-PMC causalities increased, and 3) a significant decrease in connectivity occurred from the R-PMC to R-M1. Few significant changes were seen between mild and moderate disease, except for increased bidirectional right-hemispheric cortico-cortical connectivity. Graph-theory analysis suggested that the network became more "efficient" at both local and global levels. Based on these results, it is hypothesized that: 1) premotor cortex-subthalamic nucleus interactions are altered in disease and play a role, directly or through mediating nodes, in generating excessive beta band synchrony, 2) the M1 became hyper-synchronized with the PMC, possibly leading to "locking" of neural information flow and disrupting the network's ability to desynchronize, 3) changes in modulation of R-M1 by the R-PMC may have either compensated for left hemispheric cortico-subthalamic changes or instead may have been an indirect effect of them, and 4) increased overall efficiency in the model may be related to excessive beta band synchrony induced in the Parkinsonian state. Increased understanding of connectivity within this network will hopefully increase knowledge of Parkinson's disease and improve treatments.

Disclosures: C. Toth: None. **J.C. Bore:** None. **B. Campbell:** None. **H. Cho:** None. **F. Pucci:** None. **O. Hogue:** None. **R. Gopalakrishnan:** None. **A.G. Machado:** A. Employment/Salary (full or part-time):; Cleveland Clinic - Chairman, Neurological Institute, Professor, Department of Neurosurgery. 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.; Boston Scientific Intrepid DBS trial. Funds to the institution., Enspire DBS. Funds to the institution.. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Medtronic. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Enspire DBS - distribution rights of intellectual property, Cardionomics - distribution rights of intellectual property. F. Consulting Fees (e.g.,

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Poster

P248. Parkinson's Disease: Cellular and Network Dysfunction

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P248.08

Topic: C.03. Parkinson's Disease

Support: NIH Grant R01NS079518
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Boettcher Foundation's Webb-Waring Biomedical Research Award
University of Colorado School of Medicine Center for NeuroScience

Title: Motor context modulates substantia nigra pars reticulata spike activity in patients with Parkinson's disease

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Abstract: Objectives: The severity of motor symptoms in Parkinson's disease (PD) depends on environmental conditions. For example, the presence of external patterns such as a rhythmic tone can attenuate bradykinetic impairments. However, the neural mechanisms for this context-dependent attenuation remain unknown. Here, we investigate whether context-dependent symptom attenuation is reflected in single-unit activity recorded in the operating room from the substantia nigra pars reticulata (SNr) of PD patients undergoing deep brain stimulation surgery (DBS). The SNr is known to influence motor planning and execution in animal models but its role in humans remains understudied.
Methods: We recorded SNr activity while 9 subjects (4 male; 64 ± 7 years) performed cued directional movements in response to auditory stimuli under interleaved "patterned" and "unpatterned" contexts. SNr localization was independently confirmed with expert intraoperative assessment as well as post-hoc imaging-based reconstructions.
Results: As predicted, we found that motor performance was improved in the patterned context, reflected in increased reaction speed ($p = 0.0092$, 1-tailed Wilcoxon signed rank test (paired)) and accuracy ($p = 0.0173$, 1-tailed two-sample t-test (paired)) compared to the unpatterned context. These behavioral differences were associated with enhanced responsiveness of SNr

neurons – i.e., larger changes in activity from baseline – in the patterned context. Unsupervised clustering analysis revealed two distinct subtypes of SNr neurons: one exhibited context-dependent enhanced responsiveness exclusively during movement preparation, whereas the other showed enhanced responsiveness during portions of the task associated with both motor and non-motor processes.
Conclusions: Our findings indicate the SNr serves roles in motor planning and execution, as well as warrants greater attention in the study of human sensorimotor integration and as a target for neuromodulatory therapies.

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Poster

P273. Huntington's Disease: Molecular Mechanisms I

Location: Virtual Only

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Program #/Poster #: P273.01

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH R01NS110694

Title: Alpha-synuclein and synaptic gene dysregulation in Huntington’s Disease

Authors: *A. WHITE[1], D. COATES[2], W. TSAI[1], R. GOMEZ-PASTOR[1];
[1]Neurosci., Univ. of Minnesota, Minneapolis, MN; [2]The Pennsylvania State Univ., State College, PA

Abstract: Alpha-synuclein (α-syn) is a neuronal protein that regulates synaptic vesicle trafficking and subsequent neurotransmitter release. Alteration in the levels of α-syn has been previously associated with synaptic deficits and Parkinson’s disease (PD). Recently, we and others showed that Huntington’s disease, a disease caused by a poly-glutamine expansion in the huntingtin gene, is also influenced by the levels of α-syn, although its mechanisms of action are not known. α-syn is a cytoplasmic protein but it can be found in the nucleus under pathological conditions. Our previous data showed that α-syn serine 129 phosphorylation (pS129 α-syn), a posttranslational modification associated with enhanced α-syn toxicity and PD, was increased in the nucleus of HD neurons and it correlated with synaptic changes. However, whether altered α-syn abundance and/or phosphorylation participates in synaptic gene dysregulation is unclear. To further investigate the role of α-syn in synapse regulation in HD we have taken an *in vitro* approach using mammalian cultures from immortalized striatal cells derived from control ($STHdh^{Q7}$) and HD ($STHdh^{Q111}$) mice. RT-qPCR analyses for an array of synaptic genes revealed that SNCA (α-syn) was significantly downregulated in HD cells while genes such as Slc30a3 and Slc17a7 were abnormally induced. We then manipulated the levels of α-syn by

siRNA or ectopic overexpression and found that α-syn siRNA led to a further induction of synaptic genes already upregulated in HD and up-regulation of the same genes also in control cells. On the contrary, α-syn overexpression resulted in a significant down-regulation of the exact same genes in both control and HD cells. We also generated a phospho-mimetic (S129E) and a phospho-null (S129A) α-syn mutant cell line to directly explore the effect of pS129 α-syn on α-syn's modulatory role in synaptic gene regulation. RT-qPCR of S129A and S129E expressing cells showed dysregulation of the same set of synaptic genes previously tested. These results suggest that α-syn abundance and phospho-mediated nuclear mislocalization modulates synaptic gene expression, and that alterations in synaptic gene expression observed in HD may be driven by deficits in α-syn.

Disclosures: A. White: None. **D. Coates:** None. **W. Tsai:** None. **R. Gomez-Pastor:** None.

Poster

P273. Huntington's Disease: Molecular Mechanisms I

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P273.02

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: German Research Council (DFG)
NeuroCure Cluster of Excellence

Title: Patch-seq reveals transcriptional correlates of impaired synaptic transmission in single striatal neurons in Huntington's disease

Authors: F. PARASKEVOPOULOU[1], P. PARVIZI[2], G. SENGER[2], N. TUNCBAG[2], C. ROSENMUND[1], ***F. YILDIRIM**[1];
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Abstract: Progressive transcriptional dysregulation in brain is an early and central feature of Huntington's disease (HD) pathogenesis. We and others have previously reported extensive genome-wide changes in transcription and epigenetic modifications in cell and mouse models as well as patient IPSC-derived striatal neurons. Here, using Patch-seq, a technique that combines electrophysiological recordings and single cell RNA sequencing, we studied the effects of mutant huntingtin (Htt) on transcription and synaptic transmission in single striatal neurons in an *in vitro* model of HD. Expression of mutant *Htt* decreased the synaptic output and caused dysregulation of a large number genes in single striatal neurons. Of these, we identified a group of genes whose dysregulation were correlated with the physiological deficits in neurons expressing mutant Htt. Pharmacological inhibition of histone deacetylase 1 and 3 activities rescued several functional deficits and ameliorated the aberrant gene expression in mutant Htt

neurons. Collectively, our work indicates a number of gene expression-neuronal function relationships in HD, supports an important role for epigenetic mechanisms in HD pathology and demonstrates that Patch-seq technology can be utilized to better understand molecular mechanisms of complex neurological diseases at single-cell level.

Disclosures: F. Paraskevopoulou: None. **P. Parvizi:** None. **G. Senger:** None. **N. Tuncbag:** None. **C. Rosenmund:** None. **F. Yildirim:** None.

Poster

P273. Huntington's Disease: Molecular Mechanisms I

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Program #/Poster #: P273.03

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: CIHR

Title: Wild type huntingtin reduction leads to an NMDAR-dependent loss of presynaptic sites

Authors: *J. BARRON, F. NAFAR, M. PARSONS;
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Abstract: Huntington disease (HD) results in a triad of motor, cognitive and psychiatric symptoms, and is caused by a CAG repeat expansion in the huntingtin (*HTT*) gene, which results in the production of a mutant HTT protein (mHTT). In addition to the production of mHTT, individuals living with HD also generate half the amount of wild type huntingtin (wtHTT). wtHTT has a well-known role in neurodevelopment, as its knockout is embryonic lethal in mice; however, its exact function in the adult brain is not well understood. Recent research has suggested that wtHTT plays a major role in synaptic homeostasis of adult neurons. For example, loss of wtHTT impairs presynaptic vesicle release and postsynaptic receptor localization, as well as fast axonal transport of proteins important for synaptic plasticity, such as BDNF. Long term potentiation (LTP) in the CA3-CA1 hippocampus is reliant on presynaptic glutamate release and NMDA receptor activation, as well as BDNF presence. We hypothesized that the synaptic impairments triggered by wtHTT lowering may lead to defects in NMDAR-dependent LTP. To test this, LTP was induced chemically using glycine (cLTP) in primary hippocampal cultured neurons and wtHTT levels were lowered by self-deliverable siRNA. Both presynaptic synaptophysin (SYP) and postsynaptic PSD-95 were labelled by immunofluorescence. Using super-resolution microscopy, we quantified SYP and PSD-95 puncta density from dendritic segments at the nanoscale level. cLTP induction revealed an activity-dependant decrease in SYP, but not PSD-95, density in wtHTT-lowered cells. These results signify a potential switch from synaptic potentiation to depression and subsequent loss of synapses during hippocampal NMDAR activation due to wtHTT lowering. Understanding the outcomes of wtHTT reduction in

the adult brain is critical as many HD genetic therapies in development further lower patient wtHTT levels.

Disclosures: J. Barron: None. **F. Nafar:** None. **M. Parsons:** None.

Poster

P273. Huntington's Disease: Molecular Mechanisms I

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Program #/Poster #: P273.04

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH R01NS110694
Biomedical Research Awards for Interdisciplinary New Science (BRAINS)

Title: Excitatory synapse loss and its in vivo functional correlation with ^{1}H MRS in the knock-in zQ175 mouse model of Huntington's disease

Authors: *N. ZARATE[1], K. GUNDRY[2], B. YU[1], G. OZ[2], R. GOMEZ-PASTOR[1];
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Abstract: Huntington’s disease (HD) is a devastating neurodegenerative disorder caused by a trinucleotide (CAG) repeat expansion in exon 1 of the Huntingtin gene. HD is characterized by progressive motor, cognitive, and psychiatric impairments for which there are no effective therapies or cure. Medium spiny neurons (MSNs) are preferentially affected, causing synaptic perturbations that contribute to eventual neuronal death. Previous work in cell and mouse models of HD show changes in thalamo-striatal (T-S) synaptic stability before cortico-striatal (C-S) dysfunction, motor symptom onset, or cell death. Non-invasive imaging techniques, such as ^{1}H MRS, have been used in HD mouse models and patients to monitor key neurochemical changes associated with neuronal health. However, the molecular mechanism that connects brain neurochemical changes with functional decline is still unknown, limiting our understanding of how potential treatments that may affect synapse function translate into neurochemical changes. In this study, we conducted *in vivo* longitudinal ^{1}H MRS in the striatum followed by *ex-vivo* analyses of T-S excitatory synapse density using the knock-in zQ175 HD mouse model as well as zQ175 mice lacking one allele of CK2α’(henceforth referred to as zQ175:CK2α’$^{(+/-)}$), a kinase we previously showed to be detrimental in HD. We found age- and genotype-dependent neurochemical differences between WT and HD mice, consistent with previous observations by others. Longitudinal analyses of excitatory synapse density showed a significant reduction in T-S synapses at all time points in HD mice that was rescued in the zQ175:CK2α’$^{(+/-)}$ mice. Linear regression analyses showed T-S synapse number significantly correlated with alterations in a multitude of neurochemicals and neurochemical ratios, highlighting their potential use as

surrogate biomarkers to monitor synapse dysfunction in HD. Overall, these results show the benefits of using ^{1}H MRS in future HD pre-clinical studies as a non-invasive tool to monitor the efficacy of current therapeutic trials and their effects on excitatory synaptic function.

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Poster

P273. Huntington's Disease: Molecular Mechanisms I

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Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: AG042178
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K99AG065645

Title: Mdivi 1 protects against mutant huntingtin-induced impaired mitochondrial dynamics, defective bioenergetics/biogenesis, mitophagy and synaptic damage in Huntington's Disease

Authors: *P. REDDY[1], B. RAMASUBRAMANIAN[2], N. SAWANT[2], J. PRADEEPKIRAN[2], M. VIJAYAN[2], S. KUMAR[2], H. MORTON[2], L. E. BUNQUIN[2], S. KSHIRSAGAR[2];
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Abstract: Abstract: The purpose of our study is to understand the protective effects of mitochondrial (mt) division inhibitor 1 (Mdivi 1) against mutant huntingtin (mHtt) induced mt abnormalities and synaptic damage in Huntington's disease (HD). Increasing evidence suggests that structural and functional abnormalities of mt and synaptic damage selectively found in medium spiny neurons in HD pathogenesis. Recently, we found mHtt-induced, impaired mt dynamics in striatal and cortical postmortem HD brains and BACHD and HD-knockin (HDKI) mouse models of HD. **Methods:** We treated BACHD and HDKI mice and non-transgenic wild-type (WT) mice with Mdivi 1 for six months. We performed behavioral analysis using rotarod (motor coordination), open field (locomotor activity), Y-maze (working memory) and tail suspension (neurological phenotype) in treated and untreated BACHD, HDKI (HD mice) and WT mice. Using a variety of techniques/methods, we studied cell, molecular, ultra-structural and dendritic spines in Mdivi 1 treated HD mice. **Results:** As expected, behavioral phenotype was significantly improved for all tests in Mdivi 1-treated HD mice relative to Mdivi 1-untreated HD

mice. These observations suggest that Mdivi 1 is a good compound to reduce mHtt-induced behavioral abnormalities. We also found mRNA and protein levels of mt fission genes were significantly reduced, mt fusion genes were significantly increased, mt biogenesis and synaptic genes were increased, autophagy and mitophagy genes increased. We also found increased dendritic spines in striatal, cortical, and hippocampal tissues of Mdivi 1-treated HD mice. Our electron microscopy data revealed increased mt length and reduced mt size in Mdivi 1-treated HD mice. **Conclusions:** These observations indicate that Mdivi 1 reduces mt fragmentation and enhances mitophagy and synaptic activities.

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Poster

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Program #/Poster #: P273.06

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: Taube Philanthropies
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Title: Genes related to neurotrophin signaling are associated with clinical function and brain morphology in prodromal Huntington's disease

Authors: *N. ALEXANDER, R. R. BUTLER, III, W. SYRIANI, W. A. JOHNSON, B. LEHALLIER, D. A. SIMMONS, F. M. LONGO;
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Abstract: Huntington’s disease (HD) is an autosomal dominant neurodegenerative disorder caused by a mutation that expands the length of CAG repeats in the *HTT* gene, resulting in the mutant huntingtin (mHtt) protein. mHtt disrupts diverse cellular processes leading to preferential degeneration of the striatum. Effective HD therapeutics will likely require modulation of underlying mechanisms promoting degeneration along with attempts to mitigate mHtt expression. A major contributor to HD-related degeneration is mHtt-induced loss of neurotrophic support. Neurotrophin (NT) signaling networks largely overlap those disrupted by mHtt; thus, NT receptors (primarily TrkB and $p75^{NTR}$) have emerged as therapeutic targets. As such, it is of interest to determine if NT signaling-related genes affect disease progression in prodromal HD. Accordingly, a previous study showed that *NTRK2* (gene encoding BDNF’s receptor TrkB) influenced frontal grey matter preservation in the PREDICT-HD dataset, a longitudinal clinical

study of prodromal HD. The current study evaluated how SNPs from ~100 NT signaling-related genes affect HD progression in the PREDICT-HD cohort. We examined regional brain volumes and clinical function (motor and cognitive), adjusted for CAG-age product (CAP) score, gender and genomic PCs1-10, at baseline and longitudinally—derived from functional principal component analysis (fPCA)—using genome wide association (GWA) in PREDICT-HD cases (n=929). Gene-level GWA analysis using MAGMA and H-MAGMA replicated previous associations of known residual HD age of onset-related genes (*FAN1*, *PMS1*, *MLH1*, *MSH3*). Improved SNP-to-gene mapping implicated several NT signaling-related genes—including *TRAF5, RASGRF2, PTEN* and *ADAM17*—that showed preferential association with specific HD phenotypes: grey matter of the putamen, caudate, globus pallidus, and frontal and parietal cortices, as well as cognitive and motor function (Stroop, Trail making, Single Digit Modalities tests, total functioning capacity). This genomic data paired with matching eQTL loci (in GTEx v8 and BRAINEAC databases) in corresponding brain tissues supports the role of NT signaling in residualized regional brain volumes. Replicating these analyses in larger cohorts may reveal additional NT signaling-related genomic loci of smaller effect size that contribute to the observed prodromal changes in these residual phenotypes, supporting their use as biomarkers of progression and treatment. This work provides further support for targeting NT signaling as an HD therapeutic.

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Poster

P273. Huntington's Disease: Molecular Mechanisms I

Location: Virtual Only

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Program #/Poster #: P273.07

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: CIHR

Title: Neuronal potassium-chloride cotransporter 2 (KCC2) function is altered in the indirect pathway of the basal ganglia in huntington's disease

Authors: *M. SERRANILLA, J. C. PRESSEY, M. A. WOODIN;
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Abstract: Huntington’s disease (HD) is an inherited neurodegenerative disorder characterized by progressive motor dysfunction. Motor impairments result from extensive neurodegeneration of the basal ganglia (BG), a highly interconnected assembly of subcortical nuclei best-known for their role in movement-related functions. The primary site of degeneration is the striatum, the input structure of the basal ganglia which sends inhibitory projections to the surrounding nuclei

of the basal ganglia. The striatum consists primarily of D1 and D2 dopamine receptor-expressing neurons which make up the direct pathway (dSPNs) and indirect pathway (iSPNs), respectively. dPNs and iSPNs are known to exert opposing effects on motor output, whereby activation of the direct pathway promotes movement, while the indirect pathway inhibits unwanted movement. In HD, there is a preferential loss of iSPNs, biasing basal ganglia output towards the direct pathway, though the cause of this enhanced susceptibility remains unclear. Accumulating evidence suggests that impaired synaptic inhibition within the basal ganglia circuitry may underlie this specific pattern of degeneration. Synaptic inhibition in the mature brain is largely mediated through the neurotransmitter, gamma-aminobutyric acid (GABA). GABA exerts fast hyperpolarizing inhibition by binding to Cl- -permeable GABAA receptors. GABAergic inhibition requires low intracellular Cl - concentration ([Cl-]i), which is maintained by the potassium-chloride cotransporter, KCC2. However, when KCC2 function is reduced, [Cl-]i increases, subsequently weakening the GABAergic driving force and synaptic inhibition. Usinga combination of electrophysiology, biochemical and behavioural assays, we determined that KCC2 function was reduced and GABAergic signaling was impaired in the indirect pathway of symptomatic R6/2 mice, a transgenic mouse model HD. In R6/2 mice, iSPNs demonstrated a depolarization in the reversal potential for GABA compared to dSPNs. In addition, the neuronal response to GABA was altered, from inhibitory to excitatory in the Globus Pallidus externa, the output structure of the indirect pathway. Interestingly, pharmacological reduction of [Cl-]i with the FDA-approved diuretic bumetanide effectively delayed the onset of motor impairments in R6/2 compared to controls. These findings provide evidence that the early impairments in GABAergic transmission in the indirect pathway may be a key mechanism contributing to circuitry and motor defects in HD. As therapeutic treatments for HD are limited, this work demonstrates the use of KCC2 as a novel potential therapeutic target in the treatment of HD.

Disclosures: M. Serranilla: None. **J.C. Pressey:** None. **M.A. Woodin:** None.

Poster

P273. Huntington's Disease: Molecular Mechanisms I

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Program #/Poster #: P273.08

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Grant NS111202

Title: Dynamic analysis of huntingtin interactomes in response to proteotoxic stress

Authors: *J. WEI[1], H. XU[1], E. BELIARD[1], E. CAPOBIANCO[2], M. LU[1];
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Abstract: Huntingtin protein (Htt) is a large protein (~350KDa) whose exact function is unknown. Mutation in this protein causes Huntington's disease (HD), a devastating autosomal dominant fatal neurological disorder with no cure. It is critical to understand the functions of normal Htt and mutant Htt in order to decipher the molecular pathogenesis of HD. It is strongly suggested that Htt serves as a scaffold protein that regulates protein complexes formation for various purposes. Mutant Htt is implicated in perturbing protein-protein interactions (PPI) in association with normal Htt. However, given the pathological role of cellular stresses in HD, how Htt interactome changes in response to cellular stresses remains largely unknown. To identify the dynamic interacting partners, for both normal and mutant Htt, in response to cellular stresses, we established an ascorbate peroxidase (APEX2)-based proximity labeling platform to spatiotemporally label Htt-interacting proteins in live cells. We first generated stable clones expressing Flag-APEX2-NES (nuclear export sequence, NES), Flag-APEX2-fHtt23Q (Htt23Q) or Flag-APEX2-Htt145Q (Htt145Q) using the human neuroblastoma SH-SY5Y cell line. Since Htt is largely present as a diffusible cytosolic protein, NES samples were included as a spatial probe to identify random cytosolic proteins that are in close vicinity with APEX2 due to the nature of APEX2 labeling. To test whether the APEX2-based strategy can distinguish specific local protein interaction network for normal and mutant Htt in response to proteotoxic stress, we developed a multi-step workflow to identify and quantify the abundance of biotinylated proteins in control and MG132-treated (stress) samples by 10-plex TMT quantitative mass spectrometry (MS). We identified a total of 636 proteins from the 4 plexes. After applying stringent filtering steps, 94 proteins remained for further analysis. Our primary analysis indicated that muHtt is preferentially associated with components in the intermediate filaments. We validated some of the proteins by western blot and co-immunoprecipitation. Dynamic interaction networks of Htt/muHtt in response to proteotoxic stress are currently under construction.

Disclosures: J. Wei: None. **H. Xu:** None. **E. Beliard:** None. **E. Capobianco:** None. **M. Lu:** None.

Poster

P287. Motor Neuron Disease: Human Genetics and Cellular Mechanisms I

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P287.01

Topic: C.06. Neuromuscular Diseases

Support: NIH T32 AG00096

Title: Understanding transcriptional dysregulation in ALS using RNA sequencing and computational approaches

Authors: *F. J. ARNOLD[1], A. GROMOVA[2], S. U. MCKINSTRY[3], U. RAYCHAUDHURI[1], Y. GOU[1], A. R. LA SPADA[1];
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Abstract: Amyotrophic lateral sclerosis (ALS) is an incurable neurodegenerative disorder characterized by the progressive loss of motor neurons in the brain stem and spinal cord. While the etiology of the vast majority of ALS cases remains unclear, study of inherited forms of ALS has highlighted dysregulation of RNA metabolism as a key contributor to disease. To better understand the changes in RNA processing that occur in ALS, we are utilizing RNA sequencing (RNA-seq) and computational approaches to identify transcriptional and structural alterations to the RNA landscape in cell and animal models of ALS. RNA-seq of lumbar spinal cord from a knockin mouse model of ALS4 (caused by mutations in the RNA/DNA helicase senataxin (SETX)) revealed upregulation of long non-coding RNAs and dysregulation of genes involved in axonal integrity and immune function. Additionally, we found that upregulated genes are highly expressed in microglia, while downregulated genes are highly expressed in motor neurons. To more deeply investigate transcriptional dysregulation in these key cell types, we isolated microglia from the spinal cord of ALS4 mice and performed RNA sequencing. In parallel, we sequenced induced pluripotent stem cell (iPSC)-derived motor neurons from an ALS4 patient and a gene-corrected isogenic control line. Finally, to determine if mutations in SETX directly alter its RNA binding function, we are performing UV-crosslinking and immunoprecipitation followed by RNA sequencing (CLIP-seq) of SETX from ALS4 iPSC-derived motor neurons and an isogenic control line. Using machine learning approaches to improve existing RNA secondary and tertiary structure prediction algorithms, we aim to determine the structural motifs that mediate SETX binding.

Disclosures: F.J. Arnold: None. **A. Gromova:** None. **S.U. McKinstry:** None. **U. Raychaudhuri:** None. **Y. Gou:** None. **A.R. La Spada:** None.

Poster

P287. Motor Neuron Disease: Human Genetics and Cellular Mechanisms I

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P287.02

Topic: C.06. Neuromuscular Diseases

Title: Single-nucleus sequencing reveals enriched expression of genetic risk factors sensitises Motor Neurons to degeneration in ALS

Authors: *F. LIMONE[1], D. MORDES[1], A. COUTO[1], O. PIETILÄINEN[2], B. J. JOSEPH[1], A. BURBERRY[1], S. GHOSH[3], M. THERRIEN[4], B. STEVENS[4], S. A. MCCARROLL[3], K. EGGAN[1];

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Abstract: Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder characterised by a progressive loss of motor function. While it is known that the eponymous spinal sclerosis observed upon autopsy is the result of Cortico-Spinal Motor Neuron (CSMN) degeneration, it remains unclear why this neuronal subtype is selectively affected. To understand the unique molecular properties that sensitise deep-layer CSMNs to ALS, we performed RNA sequencing of 79,169 single nuclei from the frontal cortex of patients and controls. In unaffected individuals, we found that expression of ALS risk genes was most significantly enriched only in $THY1^+$ presumptive CSMNs and not in other cortical cell types. In patients, these genetic risk factors, as well as additional genes involved in protein homeostasis and stress responses, were significantly induced in $THY1^+$ CSMNs and a wider collection of deep layer neurons, but not in neurons with more superficial identities. Examination of oligodendroglial and microglial nuclei also revealed patient-specific gene expression changes We show microglial alterations can in part be explained by interactions with degenerating neurons. Overall, our findings suggest the selective vulnerability of CSMNs is due to a “first over the line” mechanism by which their intrinsic molecular properties sensitise them to genetic and mechanistic contributors to degeneration.

Disclosures: F. Limone: None. **D. Mordes:** None. **A. Couto:** None. **O. Pietiläinen:** None. **B.J. Joseph:** None. **A. Burberry:** None. **S. Ghosh:** None. **M. Therrien:** None. **B. Stevens:** None. **S.A. McCarroll:** None. **K. Eggan:** None.

Poster

P287. Motor Neuron Disease: Human Genetics and Cellular Mechanisms I

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Program #/Poster #: P287.03

Topic: C.06. Neuromuscular Diseases

Support: GCT 2018 ERARE INTEGRALS
ASSOCIAZIONE DINO FERRARI

Title: Micrornas expression in ipsc derived motor neurons, exosomes and cerebrospinal fluid in amyotrophic lateral sclerosis

Authors: M. MENERI[1], M. RIZZUTI[1], V. MELZI[1], D. GAGLIARDI[3], D. RESNATI[3], P. MASRORI[5], L. DIONI[1], M. LOCATELLI[3], F. BIELLA[4], N. BRESOLIN[3], P. VAN DAMME[5], M. NIZZARDO[2], ***G. COMI**[3], S. CORTI[4];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a rare multifactorial neurodegenerative disorder characterized by relentless lost of motor neurons (MNs),leading to progressive muscle paralysis and precocious death. ALS is mainly sporadic, but 10% of cases are familial. At present, ALS pathogenesis has not been completely elucidated and potential molecular biomarkers of this disease remain to be identified. MicroRNA (miRNAs) are small non coding RNA molecules with post-trascriptional regulatory role. Previous studies have shown that deregulation of miRNA expression is corelated with ALS, but the downstream pathological events associated with MNs degeneration have not yet been clarified. In this study we performed microRNA expression profile analysis of iPSC-derived MNs and related exosomes from familial patients and healthy subjects. We identified dysregulation of miR-34a, miR-335 and miR-625-3p expression in MNs and exosomes. Further, we tested the expression of key miRNAs (miR-625-3p, miR-34a-3p, miR-34a-5p, miR-335-3p and miR-335-5p) in the cerebrospinal fluid (CSF) of a cohort of 54 ALS patients with a mean age of 58.4 ± 11.5 years and 19 healthy controls with a mean age of 52.8 ± 17.4 years. CFS miR-625-3p, miR-34a-5p and miR-335-3p levels were significantly dysregulated in familial patients compared to controls; miR-625-3p levels predicted familial amyotrophic lateral sclerosis diagnosis with moderate accuracy. Furthermore, the expression of miR-625-3p and miR-34a-5p significantly differed among *C9orf72-carriers*, *SOD1-carriers* and sporadic subjects. These microRNAs regulate genes and pathways that correlate well with disease pathogenesis, suggesting that studying microRNA deregulation can contribute to confirm previously identified molecular mechanisms underlying the disease and to identify new ones. Investigating patient-derived MNs, exosomes and CSF in the same study have shed light on the common pathological mechanisms underlying MNs degeneration, confirming the relevance of miRNA modulation in ALS pathogenesis. This approach might pave the way for the identification of novel biomarkers for disease diagnosis, prognosis and response to treatment.

Disclosures: M. Meneri: None. **M. Rizzuti:** None. **V. Melzi:** None. **D. Gagliardi:** None. **D. Resnati:** None. **P. Masrori:** None. **L. Dioni:** None. **M. Locatelli:** None. **F. Biella:** None. **N. Bresolin:** None. **P. Van Damme:** None. **M. Nizzardo:** None. **G. Comi:** None. **S. Corti:** None.

Poster

P287. Motor Neuron Disease: Human Genetics and Cellular Mechanisms I

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P287.04

Topic: C.06. Neuromuscular Diseases

Title: A multi-analyte evaluation of cerebrospinal fluid biomarkers for amyotrophic lateral sclerosis

Authors: A. ALEXANDER[1], D. PERREGAUX[2], D. UNRUH[1], R. WELLER ROSKA[1], A. KUBIK[1], ***Y. NOAM**[2];
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Abstract: Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by muscle atrophy that leads to eventual paralysis and death. ALS symptoms and disease progression vary significantly from person to person and this heterogeneity poses challenges for prognosis as well as missed opportunities for personalized treatment. Thus, there is a clinical need for biomarkers that reliably track disease progression and aid in treatment evaluation of ALS patients. Various fluid-based ALS biomarkers have been proposed, and recently the axonal proteins neurofilament light (NfL) and heavy (NfH) chain have emerged as potential neurodegeneration biomarkers for ALS as well as for other neuro-pathologies. In this pilot study, we utilized an automated multi-plexed microfluidic approach to analyze cerebrospinal fluid (CSF) from ALS patients and healthy donors to determine if a combination of CSF proteins can improve biomarker utility in ALS. **Methods:** The study cohort included 15 sporadic ALS patients (ALSFS-R score 25-47) and 15 age-matched controls. A total of 20 candidate biomarkers were analyzed using the automated Ella^{TM} platform. A customized microfluidic design allowed for minimal to no cross-reactivity or interference while preserving CSF volume (<110 ul per donor). Statistical modeling was applied to determine the optimal biomarker panel. **Results and conclusion:** The most salient differences in CSF profiles were manifested as significantly elevated NfL and NfH levels in ALS patients. Median levels of NfL and NfH measured at 0.83 [IQR: 0.62-1.3] and 0.95 [0.89-1.22] ng /mL in controls, and 3.7 [1.9-9.6] and 4.0 [2.3-8.6] ng/mL in ALS patients. We also saw a modest increase in the glial marker YKL-40/CHI3L1 (Ctl: 387 [289-513], ALS: 529 [412-689] ng/mL). Principal component analysis showed distinct stratification of ALS patients from controls. To evaluate the utility of a multi-analyte panel, we built a predictive statistical model trained using a leave-one-out cross validation strategy. Comparing linear regression, logistic regression, and random forest classifier models, our logistic model performed best with 93.3% accuracy and a ROC AUC value of 1.00 [0.98-1.0, 95% CI]. This model utilized measurements of 7 analytes: CRP, NF-L, NF-H, LIPO-2, GFAP, TREM-2, and VCAM-1. Building a logistic regression model with either the NF-H or NF-L measurement alone resulted in 90.0% accuracy and ROC AUC values of 0.98 [0.94-1.0] and 0.96 [0.88-1.0], respectively. Overall, our data supports a strong biomarker utility for CSF neurofilaments in ALS, whereas an alternative 7-analyte panel provides modest improvement in accuracy.

Disclosures: A. Alexander: None. **D. Perregaux:** None. **D. Unruh:** None. **R. Weller Roska:** None. **A. Kubik:** None. **Y. Noam:** None.

Poster

P287. Motor Neuron Disease: Human Genetics and Cellular Mechanisms I

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P287.05

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant S31201

Title: Neuron-to-neuron transmission of polyGR through extracellular vesicles causes degeneration in receiving cells.

Authors: *M. CICARDI[1], D. TROTTI[2];
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Abstract: Amyotrophic lateral sclerosis (ALS) and fronto-temporal dementia (FTD) are two neurodegenerative disorders which belong to the same disease spectrum. The most common genetic cause of ALS-FTD is a hexanucleotide repetition (G_4C_2) present in the intron 1 of the C9orf72 gene. This mutation results in three main toxic events: C9orf72 protein reduction, RNA foci formation and aberrant repeat associated non-AUG translation (RAN-T). The products of RAN-T are five dipeptide repeat proteins (DPRs: polyGA, polyGP, polyGR, polyPA and polyPR). Employing a mouse model which conditionally expresses polyGR, one of the most toxic DPR, we found that a fraction of the extracellular vesicles (EVs) present in the serum encloses polyGR. EVs are double membrane organelles which are released by cells through different pathways and whose content varies according to cells' patho/physiological conditions. We thus analyzed whether neurons produce EVs loaded with RAN-T products. Indeed, DPRs were detected in neuronal EVs and glutamate stimulation was able to increase the number of polyDPR$^+$ EVs. Interestingly, we found higher percentage of polyGR$^+$ EVs compared to other DPRs. We then investigate if the internalization of polyGR$^+$ EVs elicited toxicity in recipient neurons. We treated cortical neurons with polyGR$^+$ EVs and by live imaging we found a significant reduction in cell viability compared to control EVs treated neurons paralleled by TDP-43 nuclear depletion which is hallmark of ALS-FTD pathology. When we performed *in vivo* experiments injecting polyGR$^+$EVs in mouse spinal cord we observed loss of NeuN positive cells, paralleled by astrocytes and microglial recruitment at the injection site. Treating human derived motor neurons (MNs) with polyGR$^+$ EVs and we found that only C9-ALS derived MNs show increased neurodegeneration possibly owing to polyGR$^+$ EVs induced RAN-T which we were able to measure thanks to a luciferase RAN-T reporter. In conclusion we demonstrated that polyGR reception into neurons was able to cause neuronal toxicity, TDP-43 nuclear depletion and increase in RAN-T substantiating the hypothesis that EVs mediated neuron-to-neuron spreading of polyDPRs is one of the possible pathways in which neurodegeneration spreads across the central nervous system of ALS-FTD patients.

Disclosures: M. Cicardi: None. **D. Trotti:** None.

Poster

P287. Motor Neuron Disease: Human Genetics and Cellular Mechanisms I

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Program #/Poster #: P287.06

Topic: C.06. Neuromuscular Diseases

Support: NIH DP2-NS106664
New York Stem Cell Foundation

Title: Neuronal stimulator of interferon genes pathway activation in amyotrophic lateral sclerosis

Authors: *C. MARQUES[1,3], K. DORFMAN[1,3], J. SUNG[1,3], A. HELD[1,3], M. ADLER[1,3], C. I. AGUILAR[1,3], D. H. OAKLEY[1,3,2], B. T. HYMAN[1,3], L. PETRUCELLI[4], C. LAGIER-TOURENNE[1,3], B. J. WAINGER[1,3];
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Abstract: The type-I interferons (IFN) response is a well-established innate immune response to pathogens infection. However, this response has also recently been strongly implicated in neuroinflammation and neurodegeneration in diseases including Parkinson's and amyotrophic lateral sclerosis (ALS). The type-I IFN upstream regulator, stimulator of interferon genes STING, is increasingly appreciated to instigate this type-I IFN-mediated response. While much of this work has focused on activated immune cells themselves, cell-autonomous activation of innate immune signaling pathways within neurons can also occur and may play roles in neurodegenerative diseases. However, the role of STING signaling in driving this neuronal response remains unknown and represents the aim of this study. We hypothesize that STING signaling dysregulation within neurons contributes to their degeneration in the context of ALS, a rapidly progressing and fatal disorder of the motor nervous system. Approximately 10% of ALS cases are familial and result from mutations in any of the more than 30 ALS genes, of which the most common is the chromosome 9 open reading frame 72 (*C9ORF72*) intronic repeat expansion. To determine whether STING is functional within motor neurons, we performed molecular analyses on human-induced pluripotent stem cell (iPSC)-derived neurons as well as primary mouse cortical neurons subjected to modulators of STING activity. Treatment of neurons with a STING agonist or antagonist led to an increase or decrease of type-I IFN response genes expression, respectively. Thus, our data demonstrate the presence and functional integrity of STING signaling pathway in neurons. Using human iPSC lines from a range of familial ALS patients as well as mutations introduced by gene editing to create isogenic disease and control pairs, we showed a consistent increase in STING activation within ALS iPSC-derived neurons compared to control. To validate the findings *in vivo*, we used an adeno-associated virus mouse model of C9orf72 ALS and found increased STING levels in vulnerable layer V corticospinal motor neurons. To further confirm the relevance of these findings, we demonstrated similar increased STING protein in layer 5 cortical pyramidal neurons from ALS postmortem brains

compared to either Alzheimer's or non-neurological control ones. Thus, our studies demonstrate STING pathway activation in ALS neurons, with complementary findings among iPSC, mouse, and human post-mortem studies. This project is intended to provide insight into molecular pathways involved in ALS and identify targets for the development of therapeutic strategies for ALS.

Disclosures: C. Marques: None. **K. Dorfman:** None. **J. Sung:** None. **A. Held:** None. **M. Adler:** None. **C.I. Aguilar:** None. **D.H. Oakley:** None. **B.T. Hyman:** None. **L. Petrucelli:** F. Consulting Fees (e.g., advisory boards); Expansion Therapeutics. **C. Lagier-Tourenne:** None. **B.J. Wainger:** F. Consulting Fees (e.g., advisory boards); Quralis, Apic Bio, Q-StateBiosciences.

Poster

P287. Motor Neuron Disease: Human Genetics and Cellular Mechanisms I

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P287.07

Topic: C.06. Neuromuscular Diseases

Support: A Long Swim
Les Turner ALS Foundation

Title: Als motor cortex communicate their disease state very early in disease via exosome mediated signals

Authors: *M. GAUTAM[1], E. F. XIE[1], J. BRENT[1], N. KOCAK[1], *O. GOZUTOK MOORE[1], H. SHIN[1], A. GUNAY[1], M. PLEBANEK[2], C. THAXTON[2], P. OZDINLER[1];
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Abstract: Exosomes are small vesicles that transport important bio-molecular cargo (e.g. protein, RNA, miRNA and lipids) to recipient cells. They are a novel mediator of intercellular communication between different cells and organs in the body. Their ability to cross the blood brain barrier enables flow of information between central nervous system (CNS) and the periphery. Cortical dysfunctions are emerging to occur prior to symptom onset and one of the contributing factors for the initiation and progression of disease pathology in amyotrophic lateral sclerosis (ALS). This study was designed to reveal how ALS cortex communicate their diseased state via exosomes, and to reveal exosomal protein cargo that mediate the warning signal. The mice that develop ALS due to mSOD1 mediated toxicity and TDP-43 pathology are utilized together with wildtype healthy littermates. Exosomes secreted by cultured dissociated motor cortex obtained from diseased and healthy mice were isolated after 10 days of *in vitro* culture. Proteomic analysis of those exosomes suggested the presence of a common message sent out both by $hSOD1^{G93A}$ and $prpTDP\text{-}43^{A315T}$ motor cortex, which is modulated by a key transcription

factor. We performed gene modulation
experiments in both healthy and diseased neurons to investigate the impact of this transcription factor on neuronal survival. We find that the motor cortex of both hSOD1^{G93A} and prpTDP-43^{A315T} mouse models of ALS utilize exosomes to communicate their disease state even at postnatal day 3 (P3). In an effort to investigate whether this message is "protective" or "detrimental", we performed gene modulation. Our results suggest that proteins present in these exosomes appear to signal a message that codes for protection from upcoming neurodegeneration at this early age. This study unravels how cortex is involved in ALS early in the course of disease, and how exosomes can modulate key cellular events that may be targeted to overcome neurodegeneration.

Disclosures: M. Gautam: None. **E.F. Xie:** None. **J. Brent:** None. **N. Kocak:** None. **O. Gozutok Moore:** None. **H. Shin:** None. **A. Gunay:** None. **M. Plebanek:** None. **C. Thaxton:** None. **P. Ozdinler:** None.

Poster

P287. Motor Neuron Disease: Human Genetics and Cellular Mechanisms I

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P287.08

Topic: C.06. Neuromuscular Diseases

Support: the Blazer foundation

Title: Roles of MFN2 in mitochondrial transport of human spinal motor neurons

Authors: *Y. MOU[1], J. DEIN[3], Z. CHEN[1], X.-J. LI[2];
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Abstract: Charcot-Marie-Tooth (CMT) disease is one of the most common genetically inherited group of motor and sensory neurological disorders, with a global prevalence of 1 in 2500 people. CMT type 2A is one of axonal subgroups of CMT and is caused by dominant mutations in the mitofusin-2 (*MFN2*) gene. MFN2 is located in the outer mitochondrial membrane and regulates mitochondrial fusion, playing a critical role in the maintenance of normal neuronal functions. Although loss of MFN2 induces axonal neuropathy, the detailed mechanism by which MFN2 deficiency causes axonal degeneration of human spinal motor neurons remains largely unknown. In this study, we established MFN2-knockdown human embryonic stem cell (hESC) lines using lentivirus expressing MFN2 short hairpin RNA (shRNA). Using these hESC lines, we found that MFN2 deficiency did not alter the differentiation of spinal motor neurons from hESCs but induced fragmented mitochondria with abnormal function determined by live-cell imaging. Notably, MFN2-knockodwn spinal motor neurons recapitulated CMT2A disease related

phenotypes, including extensive perikaryal inclusions of phosphorylated neurofilament heavy chain (pNfH), accumulated axonal swellings, and increased pNfH levels in long-term cultures of spinal motor neurons. Importantly, MFN2 deficit impaired both anterograde and retrograde mitochondrial transport within axons by disturbing kinesin and dynein motor protein-associated gene expression. Taken together, our data reveal that MFN2 deficiency results in axonal degeneration and impairs mitochondrial morphology and function in human spinal motor neurons. The impaired mitochondrial transport in MFN2-knockdown spinal motor neurons is partially mediated by aberrant expression of motor proteins, which provides potential therapeutic targets for rescuing axonal neuropathy of spinal motor neurons in CMT2A disease.

Disclosures: Y. Mou: None. **J. Dein:** None. **Z. Chen:** None. **X. Li:** None.

Poster

P287. Motor Neuron Disease: Human Genetics and Cellular Mechanisms I

Location: Virtual Only

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Program #/Poster #: P287.09

Topic: C.06. Neuromuscular Diseases

Support: The Answer ALS Foundation
The Robert Packard Center for ALS Research

Title: Answer ALS: A Large-Scale Resource for Sporadic and Familial ALS Combining Clinical Data with Multi-Omics Data from Induced Pluripotent Cell Lines

Authors: *E. BAXI[1], T. THOMPSON[3], B. LANDIN[4], S. J. KOLB[5], S. AJROUD-DRISS[6], R. BALOH[7], D. HEITZMAN[9], T. M. MILLER[10], J. GLASS[11], R. NOREL[12], N. J. MARAGAKIS[13], M. CUDKOWICZ[14], J. VAN EYK[15], D. SAREEN[8], S. FINKBEINER[16], E. FRAENKEL[17], J. BERRY[14], L. THOMPSON[18], C. SVENDSEN[7], J. D. ROTHSTEIN[2];
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Abstract: Answer ALS was conceived and organized as a comprehensive multi-omics approach to ALS to ascertain, at a population level, the various clinical-molecular-biochemical subtypes of ALS. This national program enrolled a combined total of 1047 ALS and ALS/FTD patients and

matched control subjects. Individuals were recruited at 8 national ALS centers and followed longitudinally over one year. A smartphone-based system was employed to collect deep clinical data including fine motor activity, speech, breathing and linguistics/cognition. In collaboration with IBM Research, analytics of speech pattens reveals a strong correlation between clinical progression indices and speech. In parallel, iPS motor neurons were derived from each patient and these cells underwent multi-omic analytics including whole genome sequencing, transcriptomics, ATAC-Seq and proteomics. HIPAA compliant cloud databases were employed to store all data. Open access to all clinical, biological and molecular data is central to the program as well as public release of all generated iPS cell lines The intent of this data is for the generation of integrated clinical and biological signatures using bioinformatics, statistics and computational biology to establish patterns that may lead to a better understanding of the underlying mechanisms of disease including subgroup identification. A web portal for open source sharing of all data was developed for widespread community-based data analytics. This community-based clinical and science program provides for the identification of distinct reliably identifiable subgroups among the sporadic and familial patients and the great utility in iPS based approaches to disease pathophysiology and therapy discovery.

Disclosures: E. Baxi: None. **T. Thompson:** None. **B. Landin:** None. **S.J. Kolb:** None. **S. Ajroud-Driss:** None. **R. Baloh:** None. **D. Heitzman:** None. **T.M. Miller:** None. **J. Glass:** None. **R. Norel:** None. **N.J. Maragakis:** None. **M. Cudkowicz:** None. **J. Van Eyk:** None. **D. Sareen:** None. **S. Finkbeiner:** None. **E. Fraenkel:** None. **J. Berry:** None. **L. Thompson:** None. **C. Svendsen:** None. **J.D. Rothstein:** None.

Poster

P287. Motor Neuron Disease: Human Genetics and Cellular Mechanisms I

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Program #/Poster #: P287.10

Topic: C.06. Neuromuscular Diseases

Title: Identification of novel environmental inducers of TDP-43 aggregation linked to Amyotrophic Lateral Sclerosis

Authors: *G. FRAGOLA[1], J. M. WOLTER[2], T. J. COHEN[1];
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Abstract: Pathological aggregation of Transactive response DNA binding Protein 43 kDa (TDP-43) is a hallmark of Amyotrophic Lateral Sclerosis (ALS), a devastating neurodegenerative disease affecting upper and lower motor neurons. The overwhelming majority of ALS cases is sporadic, and the etiology likely stems from the interaction between age, genetics, and the environment. Recently, many environmental factors, including potentially damaging toxicants, have been linked to ALS through epidemiological studies, but a comprehensive identification of

all risk compounds that we as humans are exposed to has not been done. There is, therefore, an urgent need to identify environmental toxins causing TDP-43 aggregation. To address this need, we performed a high-content imaging screen by exposing cells expressing a GFP-tagged TDP-43 to a library of over 1,000 environmental compounds with high potential for human and ecosystem exposure and denoted as "at risk". This screen led to the identification of novel inducers of TDP-43 aggregation, which were validated in mouse primary neurons and in hiPSC-derived neurons. Mechanistic studies indicate that these novel compounds induce TDP-43 aggregation via new cellular and biochemical pathways and highlight unanticipated targets for therapeutic intervention. Moreover, the detection of these environmental risk compounds identifies human populations at high risk of developing ALS and other TDP-43 related diseases.

Disclosures: G. Fragola: None. **J.M. Wolter:** None. **T.J. Cohen:** None.

Poster

P287. Motor Neuron Disease: Human Genetics and Cellular Mechanisms I

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P287.11

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

Support: VA Merit BX003625 to SD

Title: Dissecting vulnerable cell types in C9orf72-associated ALS and FTD by single nucleus transcriptomic and chromatin accessibility studies of the human primary motor and medial prefrontal cortices

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Abstract: Expansion of a GGGGCC repeat in the 5' region of the first intron of chromosome 9 open reading frame 72 (C9orf72) gene is the most common genetic cause of two major neurodegenerative diseases, amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). This mutation accounts for ~40% of familial ALS, ~25% of FTD as well as ~7% of sporadic ALS cases. Precise dissection of the cell-type-specific pathophysiological alterations in C9orf72-associated neurodegenerative disease may help illuminate disease mechanisms. However, most molecular, genomic, and epigenomic studies have used bulk brain specimens that represent mixed signals from diverse neuronal and glial cell types. Here, we profiled the cell type specific transcriptome and epigenomes in two brain regions, primary motor cortex (MCX) and medial prefrontal cortex (mPFC), using post-mortem tissue samples from controls (n=6), and

patients with C9orf72-ALS (n=6) and C9orf72-FTD (n=6). We used single-nucleus RNA sequencing (snRNA-seq) and assay for Transposase-Accessible Chromatin using sequencing (snATAC-seq) to identify transcriptomic and chromatin accessibility disruptions relevant to this mutation in specific cell types. We obtained high-quality molecular profiles from ~100k nuclei in snRNA-seq and ~120k nuclei in snATAC-seq, and identified distinct classes and subtypes of neuronal and glial cells in the two brain regions. We found that the transcriptome was most disrupted by both diseases in astrocytes and subtypes of excitatory neurons. Transcriptome alterations were accompanied by concordant changes of chromatin accessibility in the gene body or distal linked enhancers of differentially expressed genes. We confirmed these findings in purified populations of the major brain nuclei obtained by fluorescence activated nuclei sorting (FANS), as well as using protein level measurements for selected differentially expressed genes. In summary, our study represents the first comprehensive characterization of molecular alterations in C9orf72-associated ALS and FTD at the level of single brain cells. Our unexpected finding of considerable ALS/FTD-associated pathology in astrocytes suggests novel biological pathways and genes that are dysregulated in these diseases.

Disclosures: M.K. Jaiswal: None. **J. Li:** None. **J. Chien:** None. **D. Lee:** None. **A. Kozlenkov:** None. **V.V. Belzil:** None. **E.A. Mukamel:** None. **S. Dracheva:** None.

Poster

P389. Treatments for Persistent Pain II

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Program #/Poster #: P389.01

Topic: D.03. Somatosensation – Pain

Support: the Medical Research Service and Rehabilitation Research Service
Department of Veteran's Affairs
PVA Research foundation
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Taylor Foundation for Chronic Diseases
VA Merit Award (I01RX002969)

Title: Targeting H-Reflex Hyperexcitability after SCI with Repurposed PAK1-Inhibitor, Romidepsin

Authors: *S. KAUER, C. BENSON, M. L. REIMER, S. PATWA, S. LIU, S. G. WAXMAN, A. M. TAN;
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Abstract: Spasticity is a clinical symptom of hyperexcitability within the spinal stretch reflex system (or H-reflex), which presents as a velocity-dependent increase in tonic stretch reflexes with exaggerated tendon jerks (Lance,1980). Individuals living with spasticity may have difficulty engaging in physical rehabilitation, experience concomitant muscle-joint pain, and experience negative quality-of-life issues (Holtz et al., 2017). Over the past decade, we have documented a common structural motif of Rac1-PAK1 regulated dendritic spine morphology associated with hyperexcitability disorders, including spasticity (Bandaru et al., 2015; Zhao et al., 2016). In this study, we investigate the utility of "repurposing" romidepsin, a clinically available drug to disrupt PAK1, to relieve spasticity after SCI. Romidepsin is a potent HDAC inhibitor that reduces PAK1 activity without affecting its protein level. Importantly, we and others have previously highlighted PAK1 as a promising druggable target for chronic neurological disease. In agreement, we have shown that romidepsin treatment has a predictable effect upon cellular and structural correlates of chronic pain (Guo et. al., 2018). To assess the efficacy of romidepsin for addressing spasticity, we will use reporter mice expressing fluorescent YFP (Thy1-YFP). To induce spasticity, we will perform a mild contusion SCI using the Infinite Horizon (IH) impactor device. Romidepsin or vehicle will be administered as three intrathecal injections four-weeks after SCI or Sham surgeries. Blinded observers will perform all physiological and behavioral assessments for spasticity and locomotor function. To analyze protein expression levels for drug response, i.e., PAK1 activity, we will process spinal cord tissue using clearing methods. We will identify a-motor neurons using established morphological criteria (Bandaru et al., 2015; Tan et al., 2012). To determine the efficacy of romidepsin treatments on SCI-induced spasticity, anatomical and biochemical data will be correlated with spasticity outcomes. YFP-expressing dendritic spine morphology will be profiled in association with H-reflex excitability indicative of spasticity. To monitor *in vivo* bioavailability, we will use assess drug-response biomarkers from all animals before and after treatment with romidepsin. A positive romidepsin drug-response in tissue and reduced dendritic spine dysgenesis that correlates with reduced spasticity outcome will support our hypothesis regarding the efficacy of targeting PAK1 activity after SCI.

Disclosures: S. Kauer: None. **C. Benson:** None. **M.L. Reimer:** None. **S. Patwa:** None. **S. Liu:** None. **S.G. Waxman:** None. **A.M. Tan:** None.

Poster

P389. Treatments for Persistent Pain II

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Program #/Poster #: P389.02

Topic: D.03. Somatosensation – Pain

Support: U.S. Dept. of Veterans Affairs Rehabilitation Research Merit Review Award B9253-C

NIH/NIGMS MSTP T32GM007205
Yale Stem Cell Center, Lo Graduate Fellowship for Excellence in Stem Cell Research
European Union Horizon 2020 No. 721841

Title: Dynamically clamping human somatosensory neurons quantifies the roles of $Na_V1.8$ and $Na_V1.9$ in electrogenesis

Authors: *M. ALSALOUM[1,2], J. I. R. LABAU[1,2], S. LIU[1,2], M. R. ESTACION[1,2], P. ZHAO[1,2], F. B. DIB-HAJJ[1,2], S. G. WAXMAN[1,2];
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Abstract: The inhibition of voltage-gated sodium (Na_V) channels in somatosensory neurons presents a promising novel modality for the treatment of pain. However, the precise contribution of these channels to neuronal excitability, the cellular correlate of pain, is unknown; previous studies using genetic knockout models or pharmacologic block of Na_V channels have identified general roles for distinct sodium channel isoforms, but have never quantified their exact contributions to these processes. To address this deficit, we have utilized dynamic clamp electrophysiology to inject precisely calibrated levels of $Na_V1.8$ and $Na_V1.9$ currents into induced pluripotent stem cell-derived sensory neurons (iPSC-SNs), allowing us to quantify how graded changes in these currents affect neuronal excitability and electrogenesis. We quantify and report direct relationships between $Na_V1.8$ current density and action potential half-width, overshoot, and repetitive firing. We additionally quantify the effect varying $Na_V1.9$ current densities have on neuronal membrane potential and rheobase. Furthermore, we examined the simultaneous interplay between $Na_V1.8$ and $Na_V1.9$ on neuronal excitability. Finally, we show that minor biophysical changes in the gating of $Na_V1.8$ can render human iPSC-SNs hyperexcitable, in a first-of-its-kind investigation of a gain-of-function $Na_V1.8$ mutation in a human neuronal background.

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Poster

P389. Treatments for Persistent Pain II

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Program #/Poster #: P389.03

Topic: D.03. Somatosensation – Pain

Support: Congressionally Medical Directed Research Program W81XWH-19-1-0673
College of Pharmacy of the University of Minnesota

Title: Non-opioid treatments for neuropathic pain: PK/PD of strategically substituted agmatines

Authors: *B. CLEMENTS[1], C. PETERSON[2], K. KITTO[2], G. L. WILCOX[3], C. A. FAIRBANKS[4];
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Abstract: Agmatine, an arginine metabolite, has been shown to reduce chronic pain in mice in a GluN2B receptor subunit-dependent manner, without motor side effects that are common for NMDA receptor antagonists. Various biopharmaceutical features, including short serum half-life, residence time, and limited blood brain barrier penetrance, challenge the utility of systemic delivery of agmatine. To address these limitations, we developed a series of substituted agmatines (SSAs), to increase lipophilicity and distribution to the CNS. We hypothesize that, relative to agmatine, SSAs reduce chronic pain with greater potency or efficacy with more ideal pharmacokinetic parameters. Assessment of the reduction of chronic pain behaviors was performed in male and female Sprague-Dawley rats following spared nerve injury (SNI). Tactile hypersensitivity was measured using von Fey monofilament stimulation. Intravenous strategically substituted agmatines dose-dependently reduced tactile hypersensitivity in nerve-injured subjects. Additionally, a reduction was seen at doses with no agmatine activity. Pharmacokinetic studies were performed in male and female rats, with serial blood collection via jugular catheters. Plasma was analyzed using LC-MS/MS, following novel analytical methods developed in our laboratory. At a dose of 13 nmol/kg, IV SSA-3 shows 2-phase distribution and elimination, with an elimination half-life of 36.7 ± 3.95 minutes. IV SSA-4 (13 nmol/kg) shows a similar distribution and elimination profile, with an elimination half-life of 38.3 ± 3.45 minutes. These data show a greater plasma half-life as compared to agmatine (9-15 minutes). When delivered orally in 5% DMSO, SSA-3 demonstrated oral bioavailability at 22.9%. Taken together we have observed that strategically substituted agmatines reduce tactile hypersensitivity arising from spared nerve injury with more ideal pharmacokinetic features.

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Poster

P389. Treatments for Persistent Pain II

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Program #/Poster #: P389.04

Topic: D.03. Somatosensation – Pain

Support: NIH Grant 5R00AT008238-04

Title: Mindfulness meditation-induced stress reductions predict pain relief

Authors: *J. DEAN[1], G. POSEY[2], G. RIEGNER[1], L. KHATIB[1], V. OLIVA[1], J. BAUMGARTNER[1], Y. JUNG[3], R. KRAFT[2], F. ZEIDAN[1];
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Abstract: Stress and chronic pain disorders are highly comorbid conditions wherein stress reliably exacerbates pain. The perigenual anterior cingulate cortex (pgACC) and the amygdala are uniquely associated with modulating pain and stress. However, it is unknown if and how stress and pain are modulated by mindfulness meditation training.
Forty healthy, pain-free individuals were randomly assigned to our validated four session (20 minutes/session) mindfulness-based (n = 20) or a book-listening control (n = 20) regimen. Prior to intervention training, participants were administered four noxious heat series consisting of ten, 10 second plateaus of 49°C thermal stimulation to the right calf at the baseline session. After completing their respective interventions, participants underwent blood-oxygen-level-dependent (BOLD) functional magnetic resonance imaging (fMRI) imaging during heat. Two heat series were administered during rest (pre-manipulation) followed by two additional heat series (post-manipulation) during mindfulness or rest (controls). Visual analog stress and pain ratings (0 = "no pain/stress"; 10 = "worst pain/stress imaginable") were collected after each heat series, respectively.
A one-way analysis of variance (ANOVA) revealed no between group differences in pain intensity ($p = 0.80$) or stress ($p = 0.27$) ratings during noxious heat at the baseline session. A 2 (mindfulness vs. control) X 2 (pre- vs. post-manipulation) repeated measures ANOVA found that mindfulness meditation significantly reduced pain intensity (32% decrease, $p < 0.001$) and stress (39% decrease, $p = 0.023$) ratings in the mindfulness but not the control group. A simple linear regression found mindfulness-induced reductions in stress predicted reductions in pain intensity ($p = 0.01$, $R^2 = 0.30$).
The present study demonstrates that mindfulness-induced reductions in stress were associated with lower pain suggesting overlapping psychological and neural mechanisms. We postulate that stronger mindfulness-induced pgACC-amygdala connectivity will be associated with lower stress and pain.

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Poster

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Program #/Poster #: P389.05

Topic: D.03. Somatosensation – Pain

Support: SIP-IPN-20210049

Title: N-(1-benzylpiperidin-4-yl)-4-fluorobenzamide a new compound with antihyperalgesic and atiallodynic effect in neuropathic rats

Authors: *M. DECIGA-CAMPOS[1], G. NAVARRETE-VÁZQUEZ[2];
[1]Inst. Politécnico Nacional, Mexico City, Mexico; [2]Facultad de Farmacia, Univ. Autónoma del Estado de Morelos, Morelos, Mexico

Abstract: The σ_1 receptor (σ_1R) plays a pivotal role in the transmission of pain; σ_1R are located in the plasma membrane and subcellular membranes, particularly in the endoplasmic reticulum, where they play a modulatory role in intracellular Ca^{2+} signaling. This work describes the design and synthesis of a novel analog of haloperidol: *N*-(1-benzylpiperidin-4-yl)-4-fluorobenzamide (LMH-2) as well as its antihyperalgesic and antiallodynic effect in rats with neuropathy induced by chronic constriction injury of the *sciatic* nerve (CCI). LMH-2 was designed as a haloperidol analog, its structure was characterized by spectroscopic (^{1}H and ^{13}C NMR spectra) and spectrometric mass (electronic impact) techniques. Also, *in silico* predictions of pharmacokinetic, pharmacodynamic, and toxicological properties were obtained with promising results. The *in vivo* antihyperalgesic and antiallodynic activity was carried out using Wistar rats with CCI. Compound LMH-2 presented a high affinity for the σ_1R in an *in vitro* binding assay, showing a K_i = 6.0 nM and high σ_1R/σ_2R selectivity ratio (>30). Subsequently, molecular docking studies were carried out to determine the binding energy and to analyze LMH-2-protein interactions. Throughout an *in silico* pharmacological consensus analysis, LMH-2 was considered safe to evaluate rats with neuropathic pain. The dose-response curves of the antiallodynic and antihyperalgesic effects showed less antihyperalgesic and antiallodynic response with respect to Gabapentin used as a positive control. However, when comparing the potency of the effects it can be seen that the new designed compound LMH-2 is more potent than gabapentin because its ED_{50} is lower with respect to gabapentin, both in the antihyperalgesic effect (LMH-2, ED_{50} = 14.8 ± 2.1 mg/kg and GBP, ED_{50} = 29.9 ± 1.1 mg/kg) and the antiallodynic effect (LMH-2, ED_{50} = 9.0 ± 1.4, GBP, ED_{50} = 35.3 ± 2.3 mg/kg) this means that a lower dose is required for LMH-2 generate the same antihyperalgesic and antiallodynic response as gabapentin in the ICC model. LMH-2 administration produces antihyperalgesic and antiallodynic effects that probably result from an antagonism at σ_1R. LMH-2 may have potential as an analgesic drug in neuropathic pain.

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Poster

P389. Treatments for Persistent Pain II

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Program #/Poster #: P389.06

Topic: D.03. Somatosensation – Pain

Support: MR141337 and PR181740 from the Department of Defense
College of Pharmacy of the University of Minnesota

Title: Strategically-substituted agmatines prevent the development of oxycodone self-administration

Authors: *C. PETERSON[1], C. BARAJAS[2], K. KITTO[1], G. WILCOX[1], C. FAIRBANKS[3];
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Abstract: The glutamatergic system is shown to play a central role in opioid addiction, therefore its inhibition has been shown to prevent the development of opioid-seeking behavior in a rodent model of drug-seeking. A decarboxylated form of L-arginine, agmatine, has been demonstrated to preferentially antagonize GluN2B-containing NMDA receptors. We have designed a series of strategically-substituted agmatine compounds (SSA1-SSA4) with the goal of improving several biopharmaceutical features of the parent compound. To this end, we have evaluated these compounds as a more viable potential therapeutic for the treatment of opioid use disorders (OUD) and evaluated their risk of dependence with repeated administration. Self-Administration: Mice (F, 21-30g) were given access to operant chambers and allowed to press either for oral oxycodone reward or an inactive control. Daily, the mice were given either a pre-treatment of a strategically-substituted agmatine (SSA3) compound or vehicle control, i.p.. Daily lever-pressing was recorded as an indication of drug-seeking behavior. Additionally, this same cohort was allowed to lever press for food pellet reward following conclusion of oxycodone reinforcement. Area under the curve (AUC) was compared between SSA pre-treated and vehicle pre-treated groups. Pre-treatment with the SSA3 compound significantly decreased oxycodone seeking behavior, but had no impact on food-maintained responding. Additionally, SSA3 showed no impact on motor coordination or cardiovascular function, side effects of concern for glutamatergic compounds. Physical dependence: Rats (M/F, 200-380g) were injected twice daily for 28 days with only one of the following compounds: SSA1-SSA4, ketamine as an in-class control, morphine as a positive control, or vehicle. The rats were observed for an hour following each injection for behavioral alterations indicative of dependence. While morphine and ketamine developed symptoms consistent with dependence associated with their respective receptor classes, the SSA compounds showed no indication of overt dependence behaviors representative of either class of compound. These data indicate that a strategically-substituted agmatine compound is capable of significantly reducing opioid-seeking behavior with a wide therapeutic window, avoiding the motor impairment and cardiovascular impairment typical of drugs of this class.

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Poster

P389. Treatments for Persistent Pain II

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Program #/Poster #: P389.07

Topic: D.03. Somatosensation – Pain

Support: NIH Grant U18 EB029257

Title: Approaches for optimizing temporal patterns of spinal cord stimulation (SCS) for treatment of chronic pain

Authors: *K. M. LAMBERT[1], T. ZHANG[2], J. GILBERT[1], M. MOFFITT[2], W. M. GRILL[1]; [1]Biomed. Engin., Duke Univ., Durham, NC; [2]Boston Scientific Neuromodulation, Valencia, CA

Abstract: Spinal cord stimulation (SCS) is an effective therapy for chronic pain used to treat tens of thousands of patients each year. There remain opportunities to improve SCS efficacy, as, in aggregate, SCS reduces pain scores by more than 50% in only ~60% of recipients, and recent clinical studies suggest that patterns of SCS beyond tonic stimulation may improve outcomes. Thus, we hypothesized that optimized temporal patterns of SCS may produce greater suppression of pain and greater reductions in spinal nociceptive neuron firing than frequency matched tonic SCS. We pursued two complementary approaches to produce optimized temporal patterns of stimulation - dual frequency SCS (dfSCS) and model-based optimization of patterned SCS. In both cases, we used a biophysical model of the dorsal horn network to test the ability of patterns to inhibit model wide dynamic range (WDR) neuron firing- a validated proxy for pain. In our dfSCS approach, two different frequencies of tonic SCS between 0-70 Hz were delivered separately to distinct populations of dorsal column axons originating from center and surround sensory receptive fields. Patterns were considered effective if they produced a 30% reduction in WDR firing rate compared to single frequency controls (higher, lower, and average) and pre-stimulation firing. The dfSCS parameter space was reduced by searching for frequency pairs that produced WDR firing rate reductions that were robust to the center-surround distribution of axons stimulated, and to loss of GABAergic and glycinergic inhibition known to occur during pain progression. Across all model states examined, the range of optimal surround frequencies was 40-70 Hz and center frequencies was 0-10 Hz. In our pattern optimization approach, we combined our computational model with a modified genetic algorithm to optimize temporal patterns of SCS that maximized a fitness function constructed to select patterns that were both more effective at reducing WDR firing rate than frequency-matched tonic SCS and more efficient than the clinical standard 50 Hz tonic SCS. In addition, patterns were evaluated for their ability to reduce WDR firing rate compared to frequency-matched stochastically generated patterns. The best performing patterns were often at average rates of ~30 Hz, with a majority of interpulse intervals between 20-30 ms or 40-50 ms, and an overall range of 1-70 ms. These patterns reduced model WDR firing rates compared to single frequency, 50 Hz, and stochastic

controls. The results demonstrate that model-based design of spatio-temporal patterns of SCS beyond tonic SCS is a promising approach to improving SCS therapy.

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Poster

P389. Treatments for Persistent Pain II

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Program #/Poster #: P389.08

Topic: D.03. Somatosensation – Pain

Support: Eyes High Postdoctoral Fellowship
Alberta Innovates mCannabis Program

Title: Cannabinoid receptor modulation of Cav3.2 voltage-gated calcium channels

Authors: *E. K. HARDING[1,2], M. A. GANDINI[1,2], T. TRANG[1,2], G. W. ZAMPONI[1,2]; [1]Univ. of Calgary, Calgary, AB, Canada; [2]Hotchkiss Brain Inst., Calgary, AB, Canada

Abstract: Cannabinoids represent a promising therapeutic avenue for treatment of chronic pain, however mixed results from both clinical and preclinical trials indicate that more work is needed to define the precise mechanism of action of cannabinoids in reducing pain symptoms. Cannabinoid receptor 1 (CB1R) is a $G_{i/o}$ coupled receptor that initiates complex signaling cascades within neurons, ultimately leading to inhibition. This occurs through interaction with a variety of proteins and ion channels, including presynaptic high voltage-activated calcium channels, which become inhibited upon CB1R activation. Low voltage-activated calcium channels such as Cav3.2 can also be present at some presynaptic terminals, including at primary afferent terminals in the superficial spinal dorsal horn, where they contribute to nociceptive processing. We therefore sought to determine whether CB1R activation by cannabinoids leads to inhibition of Cav3.2 channels. We first recorded from tsA-201 cells expressing pcDNA for Cav3.2 alone or in combination with CB1R. A time course of peak current amplitude was acquired during wash-in of the synthetic cannabinoid agonist, HU210. HU210 had no effect on Cav3.2 peak current alone up to concentrations of 5 µM. Similarly, HU210 at 1 or 5 µM did not

inhibit Cav3.2 peak current in cultures co-expressing CB1R. Positive controls performed in Cav2.2 and CB1R co-cultures showed inhibition by 1 µM HU210. We next wondered if expression of CB1R alone could affect Cav3.2 function. We found no significant differences in activation voltage or peak current density when comparing IV-curves between groups. However, comparison of steady-state inactivation curves revealed that co-expression with CB1R produced a significant ~10 mV hyperpolarizing shift in half-inactivation voltage. Our results therefore indicate that while the synthetic cannabinoid HU210 does not produce significant CB1R-mediated inhibition of Cav3.2, the expression of CB1R with Cav3.2 alone is sufficient to modulate Cav3.2 function. These results are the first indication that cannabinoid receptors can alter Cav3.2 channel gating to decrease the size of the window current. Future experiments will determine whether this is a result of constitutive activity of CB1R on Cav3.2, or a potential physical interaction between these two channels.

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Poster

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Topic: D.03. Somatosensation – Pain

Support: DoD CDMRP SCIRP SC190200

Title: Evaluation of pain reduction by RNA aptamers targeting glutamate receptors in a rat spinal cord injury model

Authors: *S. JERGOVA[1], Z. HUANG[2], A. EESWARA[1], A. PACHECO-SPIEWAK[1], L. NIU[2], J. SAGEN[1];
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Abstract: Spinal cord injury (SCI)-related neuropathic pain remains difficult to treat with poor long-term prognosis. Given the high risk of using opioid analgesics as a primary treatment, there is an urgent medical need to develop new therapeutics. Studies in diverse pain models in rats have shown that AMPA/kainate receptor antagonists can significantly inhibit pain, without abuse potential. However, properties such as nonselective off-target effects through systemic administration, and poor water solubility which reduces CNS bioavailability, are problems prevalent in virtually all existing AMPA receptor inhibitors. In this project we have tested three RNA aptamers that act as AMPA or kainate receptor antagonists as a new class of potential analgesics in a rat SCI-induced pain model. These aptamers were previously identified using

systematic evolution of ligands by exponential enrichment (SELEX). Male and female Sprague Dawley rats underwent spinal clip compression to induce SCI-pain. Animals were tested for the presence of tactile and cold allodynia at 2hrs, 1D and 4D post injection, starting at 4 week post SCI. Each of the three aptamers (FN1008, FN1040, and FB9s-b or vehicle) was injected intrathecally at a 4-day interval at the A50 dose established by prior experiments. Conditional place preference (CPP) test was performed with iv gabapentin as the conditioning analgesic drug. Potential side effects (locomotor scores, reflexes, weight) with repeated aptamer injections were monitored. Both males and female rats developed hypersensitivity to tactile and cold stimuli by week 4 post SCI. Injection of aptamer FN1008 and FB9s-b significantly reduced hypersensitivity at 2 hrs after each injection, and robust and prolonged anti-allodynic effects were observed with aptamer FN1008 and FB9s-b, as compared with weak effects of FN1040. Aptamer FN1008 and particularly FB9s-b (a kainate receptor-selective aptamer) appeared to produce more sustained anti-allodynic effects after 3-5 repeated injections that did not reverse to baseline pain levels. In addition, both aptamers reduced gabapentin CPP, suggesting a reduction of ongoing pain in treated animals. Yet, no side effects were detected from aptamer-treated rats. Interestingly, the two effective aptamers are selective for distinct receptors (FN1008 for AMPA receptors, FB9s-b for kainate receptors), suggesting blocking both AMPA and kainate receptor activities may be even more useful in pain control. Our results demonstrate that FN1008 and FB9s-b are both efficacious and safe to use and the use of RNA aptamers is a promising treatment strategy for alleviating debilitating neuropathic pain due to SCI.

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Poster

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Program #/Poster #: P389.10

Topic: D.03. Somatosensation – Pain

Support: NIH Grant GM115384

Title: A multi-region close loop BMI system for pain modulation

Authors: *G. SUN, F. ZENG, Q. ZHANG, H. XU, Y. LIU, Z. CHEN, J. WANG;
New York Univ., New York, NY

Abstract: Effective treatments for chronic pain remain limited. Conceptually, a closed-loop neural interface that combines sensory signal detection with therapeutic delivery can produce timely and effective pain relief. Such systems have been challenging to develop due to difficulty in accurate pain detection and ultra-fast analgesic delivery. Pain has sensory and affective

components, encoded in large part by neural activities in the primary somatosensory cortex (S1) and anterior cingulate cortex (ACC), respectively. Meanwhile, studies have shown that stimulation of the prefrontal cortex (PFC) produces descending pain control. Here we designed a brain-machine interface (BMI) that combines automated pain detection based on simultaneously recorded local field potential (LFP) signals from the S1 and ACC with optogenetic activation or electrical deep brain stimulation (DBS) of the prelimbic PFC in freely behaving rats. Our multi-region neural interface accurately detected and treated acute evoked pain and, more importantly, spontaneous pain associated with chronic pain. This neural interface performed with minimal delay and remained stable over time. Given the clinical feasibility of LFP recordings and DBS, our findings demonstrate the promising potential of BMI therapeutics for the treatment of pain and related neuropsychiatric diseases.

Disclosures: G. Sun: None. **F. Zeng:** None. **Q. Zhang:** None. **H. Xu:** None. **Y. Liu:** None. **Z. Chen:** None. **J. Wang:** None.

Poster

P389. Treatments for Persistent Pain II

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P389.11

Topic: D.03. Somatosensation – Pain

Support: NIDA Grant R01DA035931 to C.A.F.
College of Pharmacy of the University of Minnesota

Title: Reduction of NMDA-stimulated calcium signaling by decarboxylated arginine (agmatine) using two-photon calcium imaging

Authors: *T. XIE[1], C. D. PETERSON[2], G. L. WILCOX[3], L. VULCHANOVA[2], C. A. FAIRBANKS[4];
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Abstract: Chronic pain is a critical public health concern and effective long-term treatments are still needed. One of the targets for chronic pain management is inhibition of synaptic plasticity in the spinal cord via antagonism of the NMDA receptors (NMDAr). Specifically, antagonism of the GluN2B subunit in spinal cord dorsal horn is involved in the central sensitization and the development of chronic pain. Our previous studies have demonstrated that the intrathecal administration of agmatine, an endogenous aminoguanidine that preferentially antagonizes GluN2B-containing NMDArs, inhibits and reverses the tactile hypersensitivity without causing motor side effects. In this study, we investigated the spinal modulatory effect of agmatine at the cellular level utilizing calcium imaging. Calcium imaging is an established imaging technique to

visualize intracellular Ca^{2+} representing an indirect measure of neuronal activation, but NMDA-stimulated calcium transients have not been reported in spinal cord dorsal horn. The aim of this study is to develop NMDA-stimulated calcium transient assay in ex vivo mouse spinal cord dorsal horn and examine the effect of agmatine in this assay. Our hypothesis is that agmatine can dose-dependently inhibit NMDA-stimulated calcium transients in mouse spinal cord dorsal horn. Female and male ICR mice (4-6 weeks) were perfused before spinal cord extraction and ex vivo spinal slices were loaded with the calcium indicator dye Fluo-4. Intracellular Ca^{2+} was visualized by single plane two-photon microscopy. Time-lapse of images were acquired and the peak amplitude and AUC of Fluo-4 fluorescence intensity were analyzed by Student's t-test and ANOVA test. We observed that NMDA-stimulated calcium transients were elicited by an NMDA drug mixture containing NMDAr agonists (NMDA, glycine) and AMPA receptor antagonists (NBQX). We further demonstrated that the 5mM agmatine incubation significantly inhibited NMDA-stimulated calcium transients compared to control. Increasing agmatine concentration (0.5, 1, 2.5, 5, 10 mM) showed dose-dependent inhibition of NMDA-stimulated calcium transients. These data suggest that agmatine is an effective inhibitor of NMDArs in the spinal cord dorsal horn, and agmatine inhibited NMDA-stimulated calcium transients, consistent with our previous research demonstrating that agmatine is an effective inhibitor of NMDAr in the spinal cord dorsal horn.

Disclosures: T. Xie: None. **C.D. Peterson:** None. **G.L. Wilcox:** None. **L. Vulchanova:** None. **C.A. Fairbanks:** None.

Poster

P468. Visual System: Responses During Behavior III

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Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P468.01

Topic: D.07. Vision

Support: Intramural Research Program of the NIMH ZIAMH002958

Title: Behavioral detection of optogenetic stimulation in macaque inferior temporal cortex is influenced by visual input

Authors: E. LOPEZ[1], S. BOHN[2], R. AZADI[1], A. AFRAZ[1];
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Abstract: Anecdotal evidence from electrical stimulation of the human fusiform cortex suggests that the visual perceptual event induced by stimulation of a given cortical position varies in amplitude depending on the visual input to the eyes. This is not compatible with the intuitive view, associating a singular phosphene-like perceptual event with stimulation of a given position on the visual cortex. Here we systematically put this question to test. In two macaque monkeys,

we chronically implanted LED arrays over the central inferior temporal (IT) cortex where we had previously injected Adeno Associated Virus 5 engineered to express excitatory opsin C1V1. We also implanted the array on the opposite IT cortex where no virus injection was performed. Each behavioral trial started by fixation on a target followed by presentation of an object image randomly drawn from a set of 40 for 1 second. In half of the trials, randomly selected, a 200ms illumination impulse was delivered to the IT cortex in the middle of image presentation. Then the animals indicated if the trial contained a cortical stimulation impulse or not by looking at one of the two consequently presented targets to receive liquid reward. The two animals learned this task significantly above chance level within 17 and 8 days respectively. The analysis of results revealed a strong effect of image on the animals' ability to detect cortical stimulation ($p < 0.001$ for all) with their performance ranging from 88% to 52% for various objects. Detection of cortical stimulation was near its minimum for both animals, when no stimulus (except for a sound cue) was presented to the fixating animal during cortical stimulation (<56% for both animals). The animals were not able to detect cortical illumination over control areas with no virus transduction. In a separate experiment, we showed that detectability of optogenetic stimulation increases as the intensity of LED activation increases, and that the slope of this increase is different for various objects ($p < 0.02$ for all). Our results show that detectability of optogenetic stimulation of the IT cortex varies across different visual inputs and drops significantly when no visual stimulus is presented. This is consistent with human observations and suggests that the perceptual events induced by artificial activation of the IT cortex, highly depend on the content of vision.

Disclosures: E. Lopez: None. **S. Bohn:** None. **R. Azadi:** None. **A. Afraz:** None.

Poster

P468. Visual System: Responses During Behavior III

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Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P468.02

Topic: D.07. Vision

Support: Intramural Research Program of the NIMH ZIAMH002958

Title: Visibility of visual input strongly modulates detectability of optogenetic stimulation of inferior temporal cortex

Authors: *R. LAFER-SOUSA, K. WANG, A. AFRAZ;
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Abstract: Stimulation of visually sensitive regions of ventral temporal cortex in humans has been shown to cause changes in perception, yet the precise nature of these effects remains unclear. In some cases, cortical stimulation while an object or image is being viewed suggests an

additive effect wherein a hallucinatory image is superimposed on the image that has been streaming from the retina (e.g. Schalk et al. 2017). Other cases suggest such stimulation causes a distortion of the contents of the visual input (e.g. “your face metamorphosed, your nose got saggy and went to the left” Ragarajan et al. 2014). Clarifying the nature of these perturbations is critical to understanding the role these brain regions play in behavior and perception. In order to systematically test whether stimulation of inferior temporal (IT) cortex causes additive versus distortive effects, we carried out optogenetic stimulation in macaque monkeys performing a stimulation-detection task while viewing images of objects that varied in visibility. Hypothetically, if stimulation causes an additive effect, varying visibility of the visual input should not affect detectability of stimulation. If anything, stimulation should be easier to detect when the visual input is less visible since there would be no underlying image to parse from the hallucinatory percept. If on the other hand cortical stimulation has a distortive effect, it should be easier to detect when the visual input is more visible, as the effect would necessarily be a function of the visual input. Hence, the stimulation-detection task is well-suited to distinguish between the distortion model and the additive model. Two macaque monkeys were implanted with arrays of LEDs over a region of their IT cortex transduced with the depolarizing opsin C1V1. In each trial, following fixation an image was displayed on the screen for 1s. In half of the trials, randomly selected, an LED was turned on for 200ms half way through image presentation, and the animal was rewarded for correctly identifying whether the trial did or did not contain cortical stimulation. The image set consisted of objects that degraded in contrast, saturation, and spatial frequency to near gray in 4 steps. Visibility of the visual input had a significant effect on stimulation detectability (ANOVA, M1 $p = 0.0036$, M2 $p = 1 \times 10^{-6}$). Consistent with the distortion model, the animals were more accurate at detecting stimulation when the visual input was more visible.

Disclosures: R. Lafer-Sousa: None. **K. Wang:** None. **A. Afraz:** None.

Poster

P468. Visual System: Responses During Behavior III

Location: Virtual Only

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Program #/Poster #: P468.03

Topic: D.07. Vision

Support: 1ZIAMH002958-04

Title: Perceptography: behavior guided graphical reconstruction of phosphenes induced by electrical stimulation of the primary visual cortex

Authors: *T. MA[1], E. SHAHBAZI[1], A. BOWMAN[2], A. AFRAZ[3];
[1]Natl. Inst. of Hlth., North Bethesda, MD; [2]NIMH, Kensington, MD; [3]NIH, Bethesda, MD

Abstract: Is it possible to take a picture of a visual perceptual event induced by stimulation of the visual cortex? While the question sounds philosophically impenetrable, we have developed a concept, perceptography, to approximate such pictures; perceptograms. We define a perceptogram as an altered image, which is indiscriminable for the observer from the state of being cortically stimulated in a given visual area, while viewing the original image. To test the concept, we chose the primary visual cortex (V1) as a testbed because the perceptual events resulting from stimulation of area V1, known as phosphenes, are well studied in both humans and monkeys, yet loosely characterized in regards to their shape, timing, and luminance. A macaque monkey was trained to behaviorally detect short electrical impulses (250 μA, 40Hz bipolar square wave, 200 ms) delivered to its V1 via a Utah array while fixating at a dynamic pattern of low-frequency enhanced white noise subtending 23 degrees. Each trial lasted 1s, included 5 refreshments of the noise pattern, randomly included cortical stimulation halfway through the trial and was followed by two response targets for the monkey to indicate if the trial was "stimulated" or not. We only used stimulation in one position in the cortex for collecting each batch of data. We then marked the trials that led to a false alarm (12% of trials). We hypothesized that a false alarm is more likely if a pattern of noise happens to mimic the visual timing and features of the electrically induced phosphene. Thus, at first pass we averaged all the noise patterns that led to ~800 FAs across ~6000 behavioral trials and 3 cortical positions. For different cortical positions on V1, we found light and dark phosphenes significantly standing out of the noise (mean difference from average gray 4.6%, St.D 0.22, $p<0.01$, corrected for multiple comparisons). These phosphenes matched the location and timing of the saccades induced by electrical stimulation of their corresponding cortical position with the fixation condition relaxed. They also matched the location of the receptive field of the neurons directly under the stimulated shaft of the array.

Disclosures: T. Ma: None. **E. Shahbazi:** None. **A. Bowman:** None. **A. Afraz:** None.

Poster

P468. Visual System: Responses During Behavior III

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P468.04

Topic: D.07. Vision

Support: NIMH ZIAMH002958 (to A.A.).

Title: Perceptography, behavior guided reconstruction of visual percepts induced by stimulation of the inferior temporal cortex.

Authors: E. SHAHBAZI[1], T. MA[1], M. PERNUS[2], R. AZADI[3], W. SCHEIRER[4], *A. AFRAZ[1]; [1]NIH/NIMH, Bethesda, MD; [2]Fac. of Electrical Engin., Univ. of Ljubljana, Ljubljana, Slovenia;

[3]Natl. Inst. of Mental Hlth., Bethesda, MD; [4]Computer Sci. and Engin., Univ. of Notre Dame, South Bend, IN

Abstract: Cortical stimulation in high-level visual areas induces complex perturbations in visual perception. Understanding the nature of stimulation-induced visual percepts is necessary for characterizing visual hallucinations in psychiatric disease and for the development of visual prosthetics. While most of the evidence comes from anecdotal observations in human patients, systematic study of the topic has been severely limited in the absence of language faculty in nonhuman primates. We developed a new method, perceptography, to take pictures of complex visual percepts induced by optogenetic stimulation of the inferior temporal (IT) cortex in macaque monkeys. Each trial started with the animal fixating on the screen, followed by a 1s presentation of a natural-looking image produced by a generative adversarial network (GAN). Halfway through the image presentation, we briefly altered the image features. In half of the trials randomly selected, a ~1x1mm area of IT cortex was optogenetically stimulated, for the same duration as the image alteration, via an implanted LED array using the excitatory opsin C1V1. The animals were trained to report cortical stimulation with eye movements following the image offset. The animals learned to discriminate image feature alterations from those induced by cortical stimulation and were rewarded only for successful detection of the latter. Under the hood, two deep learning systems, DaVinci (GAN) and Ahab (Deep learning feature extraction pipeline), controlled image alterations and tracked the animals' behavioral responses, respectively. We hypothesized that false alarms (FA) are more likely to happen when an image alteration shares common features with the percept induced by cortical stimulation. In a functional closed loop with the animal, Ahab guided DaVinci to make image alterations that reduce the discriminability between stimulated and nonstimulated trials, thus increasing the chances of FA. While the baseline FA rate for nearly all image alterations remained at ~2.5%, within 10,000 trials(5 days), the pool of Ahab optimized images evolved to induce 8-10% FA. Among these were five images that induced 55-79% FA (cross-validated, $p<0.01$). We would like to call these images Perceptograms of IT, as the state of seeing them is hard for the animal to discriminate from the state of being cortically stimulated.

Disclosures: E. Shahbazi: None. **T. Ma:** None. **M. Pernus:** None. **R. Azadi:** None. **W. Scheirer:** None. **A. Afraz:** None.

Poster

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Title: Models of category-selective brain regions recapitulate key findings and enable high-throughput tests of selectivity

Authors: *N. RATAN MURTY[1], A. ABATE[1], P. BASHIVAN[2], J. DICARLO[1], N. KANWISHER[1];
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Abstract: Cortical regions apparently selective to faces, places, and bodies have provided important evidence for domain-specific theories of human cognition, development, and evolution. But these claims of category selectivity are not quantitatively precise and remain vulnerable to empirical refutation. To address these limitations, we constructed deep convolutional artificial neural network (ANN) -based encoding models that accurately predict the observed fMRI response to novel images in the FFA, PPA, and EBA, outperforming both descriptive models and experts in the field. Even though not explicitly trained to do so, the models recapitulate several key findings in the literature (eccentricity and curvilinearity bias etc.) and provide important clues about the specific image features that drive the responses in these regions. We further used these computational models to subject claims of category selectivity to their strongest tests, by screening for and synthesizing images predicted to produce the highest response in each region. We found that the high-response-predicted images were all unambiguous members of the hypothesized preferred category for each region. Together these results provide highly accurate, image-computable encoding models of each category-selective region which we provide to the community as implementable online code. The results from our experiments also strengthen evidence for domain specificity in the human brain, and point the way for future research characterizing the functional organization of the brain with unprecedented computational precision.

Disclosures: N. Ratan Murty: None. **A. Abate:** None. **P. Bashivan:** None. **J. DiCarlo:** None. **N. Kanwisher:** None.

Poster

P468. Visual System: Responses During Behavior III

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Program #/Poster #: P468.06

Topic: D.07. Vision

Support: ZIAMH002958

Title: Opto-array: a chronically implantable led array designed for behavioral optogenetic experiments in nonhuman primates

Authors: *R. AZADI[1], E. LOPEZ[1], R. RAJALINGHAM[2], M. SORENSON[3], S. E. BOHN[4], A. AFRAZ[1];
[1]NIH, Bethesda, MD; [2]Brain and Cognitive Sci., MIT, Cambridge, MA; [3]Engin., Blackrock Microsystems, Salt Lake City, UT; [4]Neurosci. Grad. Group, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Optogenetics has revolutionized the landscape of systems neuroscience by enabling the scientist to perturb neural activity with precise spatial and temporal resolution and cell-type specificity. We developed the Opto-Array, a novel chronically implantable array of LEDs that can reliably deliver light in optogenetic experiments in large brains, specifically nonhuman primates. The OptoArray contains a 5x5 grid with 24 LEDs and one thermal sensor on a PCB encapsulated with a transparent parylene and silicone coating. The 2D configuration of LEDs enables perturbation of a large range of surface areas from (~1x1mm) for only one active LED to (~5x5mm) for the entire array turned on. The thermal sensor monitors the heating of the cortical tissue from light delivery to avoid any potential damage. The chronic nature of Opto-Array allows highly stable behavioral experiments, in which data can be pooled over months. Moreover, this tool provides a safer alternative to acute methods (i.e. using optical fiber and direct illumination), minimizing the tissue damage as well as the risk of infection associated with open cranial windows and chambers. Here we explain the surgical techniques and procedures for implantation of Opto-Array in macaque monkeys.

Disclosures: R. Azadi: None. **E. Lopez:** None. **R. Rajalingham:** None. **M. Sorenson:** A. Employment/Salary (full or part-time):; Blackrock Microsystems. **S.E. Bohn:** None. **A. Afraz:** None.

Poster

P468. Visual System: Responses During Behavior III

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Program #/Poster #: P468.07

Topic: D.07. Vision

Support: NIMH 1P50MH094271
WPI-JSPS

Title: Awake functional ultrasound imaging reveals rigidity of brain dynamics in reversible Shank3 mutant mice

Authors: *T. HENSCH[1], C. ORAM[2], T. WATANABE[3];
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Abstract: Human fMRI studies suggest that autism is underpinned by aberrant brain dynamics (Watanabe & Rees, 2017). To probe these mechanisms and their potential reversibility, whole-brain imaging from awake animals is required. We established a functional ultrasound (fUS) imaging paradigm for head-restrained wildtype (WT) and Shank3-deficient mice behaving freely on a ball while monitored for arousal state by concurrent pupillometry. Long-range resting state maps revealed a significant hypo-connectivity that was more prevalent in Shank3 heterozygous mice and at low arousal state. In parallel, we expanded the energy landscape analysis used previously for human fMRI data to deal with time-series data recorded from a relatively large number of neural areas. By applying the extended energy landscape analysis to the mouse fUS data, we examined the effects of Shank3 expression on brain state dynamics. First, we identified that the genetic disruption of Shank3 significantly deteriorated the flexibility of brain state dynamics (Cohen's $d > 6.0$; $P < 10\text{-}7$) due to significantly unstable minor brain states (Cohen's $d > 1.8$; $P < 0.0003$), which is consistent with our previous study of autistic human subjects (Watanabe & Rees, 2017). Instead, the duration of major brain states was unchanged in Shank3 mutants, unlike for high-functioning autistic subjects where it was elevated in parallel with their IQ. Notably, we found that Tamoxifen-induced re-expression of Shank3 in adolescent (P22-42) mice could recover their rigid brain state dynamics by stabilising the minor brain states (Cohen's $d > 1.7$; $P < 0.0005$), while not rescuing static resting-state connectivity. Thus, fewer neural transitions due to an unstable intermediate state may predict the severity of autism in both man and mouse. More broadly, it is now possible to bridge pre-clinical models of ASD at the whole-brain level to analogous non-invasive fMRI datasets from human subjects.

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Poster

P468. Visual System: Responses During Behavior III

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Topic: D.07. Vision

Support: BCH IDDRC 1U54HD090255
CIFAR
HFSP-CDF LT000975/2015C
Rettsyndrome.org
AIRETT

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WPI-IRCN - Japan Society for the Promotion of Science

Title: Whole-brain network switching by arousal requires cholinergic MeCP2 expression

Authors: *P. ARTONI[1], C. ORAM[1], J. LEBLANC[2], V. VINCI[1], C. A. NELSON[2], M. FAGIOLINI[1], T. K. HENSCH[3];
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Abstract: Whole-brain networks continuously balance between modes of integration and segregation. While integration occurs when different brain areas work cooperatively, segregation arises as individual domains emerge to perform specific tasks. By combining large-scale functional ultrasound (fUS) imaging in awake, behaving mice during visual stimulation with network analysis, we found that arousal state transitions reversibly switch between a dorsal integration pathway and bottom-up sensory specialization. In contrast, the MeCP2-deficient mouse model of Rett Syndrome (RTT) failed to toggle their whole-brain networks efficiently. Selective restoration of MeCP2 within cholinergic (but not noradrenergic) circuits was sufficient to rescue cortical network dynamics and their associated multiplicative gain both globally and at single-cell resolution. The aberrant sensory gain trajectory in MeCP2-deficient mice was recapitulated by regression stage in RTT patients. Therapeutic strategies tracking arousal states and targeting cholinergic circuits may thus pre-empt deficits of systems-wide network dynamics in the etiology of neurodevelopmental disorders.

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Poster

P468. Visual System: Responses During Behavior III

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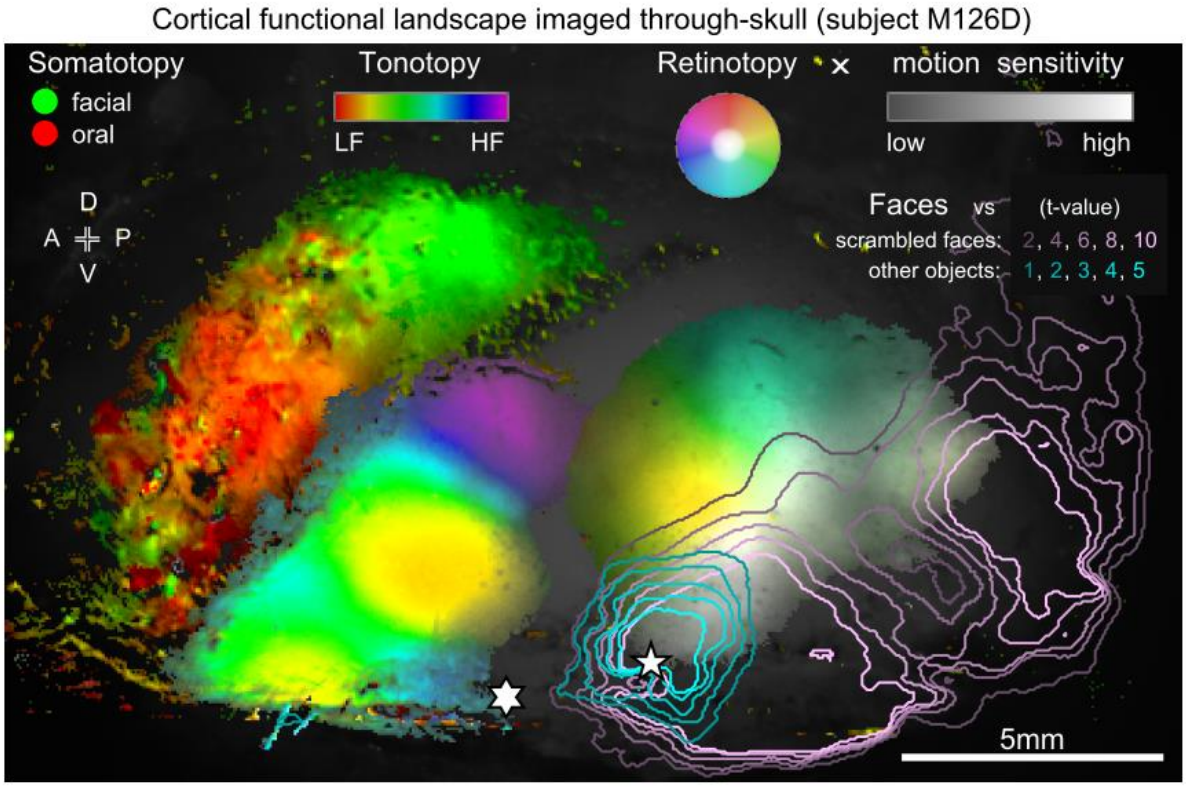
Topic: D.07. Vision

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NIH Grant DC014503
DARPA Cooperative Agreement N66001-17-2-4008

Title: Mesoscopic landscape of cortical functions revealed by through-skull wide-field optical imaging in marmoset monkeys

Authors: *X. SONG[1], Y. GUO[2], H. LI[2], C. CHEN[2], J. LEE[3], Z. SCHMIDT[2], X. WANG[3]; [1]Johns Hopkins Med. Institutions, Baltimore, MD; [2]Johns Hopkins Univ., Baltimore, MD; [3]Dept Biomed Engin, Johns Hopkins Univ. Sch. Med., Baltimore, MD

Abstract: The primate cerebral cortex is organized into specialized areas representing different modalities and functions along a continuous surface. The functional maps across the cortex, however, are often investigated a single modality at a time (e.g., audition or vision). To advance our understanding of the complex landscape of primate cortical functions, here, we developed and applied to awake primates a polarization-gated wide-field optical imaging method for measuring cortical functions through the un-thinned intact skull. In awake marmoset (*Callithrix jacchus*)—a primate species featuring a smooth cortex—adjacent auditory, visual, and somatosensory cortices were noninvasively parcellated in individual subjects with detailed tonotopy, retinotopy, and somatotopy. A new pure-tone-responsive tonotopic gradient was discovered in the auditory cortex and a face-patch sensitive to motion in the lower-center visual field was localized near an auditory region representing frequencies of conspecific vocalizations. These adjacent cortical regions may form a network coordinating audiovisual information such as vocalization sounds and month movement for vocal communication. This through-skull landscape-mapping approach opens new opportunities for understanding how the primate cortex is organized and coordinated to enable real-world behaviors.



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Poster

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Program #/Poster #: P468.10

Topic: D.07. Vision

Support: NIH Grant U01 NS116377

Title: Gaze and body tracking of freely moving marmosets for visual neurophysiology during naturalistic perception-action cycles

Authors: *V. SINGH[1], A. HUK[2], J. L. YATES[2], V. NGO[1], S. M. SHIELDS[2], C. MILLER[1]; [1]UCSD, San Diego, CA; [2]The Univ. of Texas, Austin, TX

Abstract: Recently, there has been a surge of interest to investigate behaviour in freely moving animals as brain activity in the freely-moving vs constrained (e.g., head-fixed) states may not necessarily be identical. Marmosets are an emerging animal model of choice because of their small size, fast development, exquisite visual systems, and membership in the same taxonomic order as humans. However, reliable systems for gaze and body movement tracking in freely-behaving marmosets are not yet available. Leveraging current advancements in Single-Board-Computers (SBC) and artificial neural networks (ANNs), we report methods in two distinct but integrated domains. First, we developed a novel wireless, head-mounted eye tracking system with an eye camera and a scene camera that are calibrated, allowing estimation of where the animal is looking in the scene while being freely foraging in an open arena. We then use a semantic segmentation ANN to determine the gaze dynamics. The data streams from the eye-tracking cameras are synchronized with wireless neural recording. Second, we use another ANN environment (DeepLabCut) to estimate the location and movements of the animal when the animal is hunting for food (field crickets) in a large arena with multiple cameras on each side of the room. These approaches have substantial implications for the feasibility of using freely-moving nonhuman primates in navigational studies, visual neuroscience and other domains where the inherent constraints of the head-fixed preparation limit opportunities to elucidate key dimensions of brain function. In the case of our visual neuroscience projects, this integrated set of hardware and ANN-based analysis allow for detailed, quantitative characterizations of visual processing during natural behaviors.

Disclosures: V. Singh: None. **A. Huk:** None. **J.L. Yates:** None. **V. Ngo:** None. **S.M. Shields:** None. **C. Miller:** None.

Poster

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Program #/Poster #: P468.11

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AFOSR 19RT0316

Title: Visuomotor Integration for Natural Hunting Strategies in Wild Marmosets

Authors: V. NGO[1], F. DE LA FUENTE[2], J. GORMAN[1], A. SOUTO[3], A. HUK[4], N. SCHIEL[2], *C. MILLER[1];
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Abstract: Marmoset monkeys (*Callithrix jacchus*) are prolific hunters. This arboreal New World primate employs a range of dynamic strategies tailored specifically to the distinct strategies needed to successfully prey on a diversity of different insects, ranging from terrestrial ants to flying beetles and dragonflies. Here we sought to quantify the visuo-motor integration that occurs during these different behavioral strategies for successful prey capture in wild marmosets at the Baracuhy Biological Field Station (Cabaceiras, Brazil) employ. We analyzed ~600 high-resolution video recordings of hunting events and quantified how individuals coordinate their limbs, head, and body to successfully visually identify, monitor, and attempt grasps of their target prey. Our preliminary analyses show that hunting strategies were dependent on several factors including prey size, their relative position on the substrate to the prey, the area in which they hunt, and whether conspecifics were nearby. We observed that when marmosets hunt in trees, they typically reach for prey with two hands while extending their body more than half their original body length during a coordinated ballistic grasp. While when hunting on the forest floor, their body extension was significantly less due to their body position when grasping and animals typically retrieved insects with their mouth. We are currently employing modern computer vision technology -DeepLabCut - to more accurately quantify the visuomotor integration that occurs during two of the more dynamic hunting strategies. Notably, the most frequent hunting strategy marmosets employed was a two-handed ballistic grasp following a behavioral pause for motor-planning and visual optimization in the arboreal substrate. A second strategy analyzed here involved rapid head-gaze tracking behavior of fast flying beetles which involved either one- or two-handed capture mid-air. Overall, data suggests that distinct visuo-motor integration strategies for prey capture were implemented based on the stability of the substrate and optimization of pre-attack positional behavior. The marmoset's natural prey-capture behavior offers an exciting opportunity to investigate active vision in a nonhuman primate, including the opportunity to quantify visuo-motor integration in the wild and under more controlled laboratory conditions.

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Poster

P468. Visual System: Responses During Behavior III

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Program #/Poster #: P468.12

Topic: D.07. Vision

Support: WPI-JSPS (Japan Society for the Promotion of Science)
NIMH 1P50MH094271

Title: Rapid synaptic and gamma frequency signature of critical period brain state

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Abstract: Early life experience sculpts thalamic axons carrying sensory information to the cortex. Here, we identify the thalamo-cortical (TC) targets that initiate the rewiring process which manifests in altered cortical oscillations. Monocular deprivation (MD) acutely induced a transient (<4hr) peak in EEG gamma (~40Hz) power specifically within mouse visual cortex, but only under conditions of open critical period plasticity (juvenile or adult dark-reared wildtype, adult Lynx1- or GAD65-deficient mice with diazepam). Concomitant, immediate loss of thalamic input onto fast-spiking (FS) GABA neurons (but not pyramidal cells) was observed within hours of MD using a TC slice preparation of the mouse visual pathway - again only if the critical period was open. Computational modeling of the TC pathway suggests that the emergent 40 Hz rhythm in response to MD is the result of a cortical interneuronal gamma (ING) rhythm in networks of FS neurons. Conditions for ING, including the presence of interneuronal gap junctions, appear at the start of the critical period, rapidly forming a rhythm when TC input is imbalanced by MD. The ING rhythm effectively dissociates thalamic input from cortical spiking leading to a rapid loss of previously strong TC-to-FS connections through standard spike-time dependent plasticity rules. A direct consequence of weakening the dominant TC input is that, on a slower time-scale, previously silent TC-to-FS connections are allowed to strengthen. Thus, ING enables cortical dynamics to transition from being dominated by the strongest TC inputs prior to MD to sensing the statistics of the population TC input after the networks have stabilized following MD. Our network model further captures the gradually increasing gamma frequency

and eventual fade-out of the rhythm as it evolves over time, suggesting that the fleeting ING accompanying MD can serve as a readout of the TC network state. Our findings reveal the first synaptic events underlying critical period plasticity and identify gamma oscillations induced by brief sensory challenge as a robust biomarker with which to probe disrupted developmental trajectories, such as in cognitive disorders like autism and schizophrenia.

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Poster

P468. Visual System: Responses During Behavior III

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Topic: D.07. Vision

Support: Wellcome/Royal Society Sir Henry Dale Wellcome Fellowship awarded to PK (218535/Z/19/Z)
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Title: Distinct laminar profiles in V1 for 'hallucinated' and accurately detected gratings

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Abstract: In order to make meaning of the noisy sensory world, it is thought that the brain integrates prior expectations with sensory input. Distinct cortical layers are believed to play different roles in achieving this, with agranular layers receiving feedback connections to provide contextual input, whereas the granular layers are thought to provide bottom-up sensory input. However, the exact computational roles of the distinct layers in balancing sensory inputs and internal signals remains unclear. Here we use layer-specific fMRI to study the cortical microcircuitry underlying perception and hallucination in healthy individuals. On the first day, we trained human participants (N=24) on a forced two choice perceptual discrimination task, where participants were presented with noisy grating stimuli, and had to decide whether they perceived a clockwise or anti-clockwise tilted grating, as well as report their confidence that a grating was perceived. On the second day participants performed the task while ultra-high-resolution fMRI (0.8mm) was used to measure brain activity. Importantly 50% of trials on the second day contained noise only, which allowed us to explore detection of gratings embedded in noise, as well as false percepts of gratings in noise ('hallucinations'). We found that, whilst participants were able to perform the task with high accuracy, they were unaware upon debriefing that 50% of trials did not contain gratings, with participants estimating there to be

10% of noise trials on average instead. Similarly, participants reported seeing gratings with higher-than-average confidence on 32% of noise trials. A logistic regression model revealed that previous responses and percepts, as well as an auditory cue that signalled the most likely orientation to the participant during training, contributed significantly to hallucinated gratings. Crucially, the laminar profile of perceived and hallucinated gratings in V1 was strikingly different. Specifically, we found a strong interaction between confidence and layer on stimulus trials, where high confidence responses on accurate trials was related to stronger middle layer activity. In contrast, hallucinated gratings on noise trials were particularly related to superficial layer activity. We conclude that despite subjectively similar experiences, the laminar profile of accurately detected and hallucinated gratings are distinct, where the input layers are the key-determinant in driving perceptual confidence in stimulus trials, it is the superficial layers which combine feedback activity from higher regions with input from the middle layers to create a false percept.

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Poster

P498. Large Scale Analysis of Anatomy and Population Dynamics

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Program #/Poster #: P498.01

Topic: D.07. Vision

Support: NIH Grant 5U19MH114830-03

Title: Whole-brain reconstruction of different types of single spiny neurons in the visual cortex of mice

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Abstract: Excitatory neurons are the majority in visual cortex (VC) including primary region (VC1) and high functional regions (VC2), which have been intensively studied and defined into many types in different layers using brain slices. Full neuronal morphologies are still unknown for these neuron types. To describe and classify these neurons based on complete morphologies, we performed computation-assisted morphological reconstructions to obtain full dendrites and axons of single neurons across all layers of VC using Vaa3D-TeraVR from a high resolution whole brain image stack acquired with a two-photon fluorescence micro-optical sectioning tomography system (*2p*-fMOST). We sparsely labeled neurons using tamoxifen-inducible Cre driver lines crossed to a bright GFP reporter. Based on whole brain reconstruction, we described

preliminary results for excitatory neurons in VC1 and VC2. In total, 169 neurons (89 in VC1, 80 in VC2) were reconstructed, including 18 types from layer 2 to layer 6. Tufted PCs in layers 2, 3 and 4 ***(L2TPC, L3TPC, L4TPC)*** had a balanced number of two subtypes with ipsilateral (***TPC_ips***) and bilateral (***TPC_bi***) projections to multiple cortical regions. The TPC_bi cells formed asymmetrical axonal clusters between the two hemispheres predominantly to the ipsilateral side. In layer 4, ipsilateral subtypes were predominant among neuron types studied (***L4TPCs***, ***L4UPCs***); Small tufted PCs in layer 5 (***L5SPCs***) featured long axonal collaterals along pial, and had ipsilateral and bilateral subtypes, forming multiple projections in the cortex. ***L5SPC_bi*** cells were the predominant type in VC2 and formed asymmetrical richer axonal clusters between two hemispheres. They projected predominantly in the ipsilateral side, which often projected to a subcortical region. Featured by thick tufted apical dendrites with a later bifurcated tuft, simple L5TPC cells (***L5TPC_sim***) were the only thick tufted PC type in VC1 and the predominant type in VC2 where complex L5TPCs (***L5TPC_com***, with an early bifurcated tuft) were encountered occasionally. A narrow PC type in layer 6 (***L6NPCs,*** L6CT cells) in both VC1 and VC2 consistently had a single projection to thalamus but targeted different subregions. Featured with two big dendrites, bipolar PCs in layer 6b (***L6bBPCs***) also have ipsilateral and bilateral subtypes forming multiple projections in the cortex. ***L6bBPC_bi*** neurons had symmetrical axonal arbors between the two hemispheres. These results indicate that individual excitatory neuron types in VC typically have ipsilateral and bilateral subtypes; their soma locations and local dendritic and axonal morphologies are correlated with their axonal projections at whole brain level.

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Poster

P498. Large Scale Analysis of Anatomy and Population Dynamics

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Program #/Poster #: P498.02

Topic: H.03. Decision Making

Support: Wellcome Trust (216324/Z/19/Z)
Simons Foundation

Title: Whole dorsal cortex dynamics and behavioral correlates in the International Brain Lab task

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Abstract: Making a decision is a complicated process that includes processing current sensory information, integrating the current information with prior information, planning a motor output, and executing that motor command. Prior information has a strong influence over decision-making behavior. However, how prior information influences the neural activity underlying the different processes in decision-making remains unclear. Here we use the task standardized by the International Brain Laboratory (IBL) to investigate the neural basis of how sensory evidence is combined with prior information in the process of making decisions. The IBL task requires head-fixed mice to report whether a visual stimulus appears on the left or right side of a screen by turning a wheel left or right. In addition to this simple decision task, the prior probability of the stimulus appearing on one side switches, in blocks of about 50-100 trials, from 80% on one side, to 80% on the other. The IBL as a whole is investigating the brain-wide correlates of this task in single-unit neuropixels probe recordings. However the number of simultaneously recorded areas across the brain is relatively small in such recordings. Here we have used an alternative physiological approach to assess neural activity in many brain areas simultaneously, in order to probe how these areas represent and integrate the neural activity underlying this behavior. We used widefield GCaMP6s calcium imaging to record the activity of the whole dorsal cortex during task performance. We find neural activity in general to be highly correlated across the brain. These correlations arise through a combination of extrinsic (task-associated) and intrinsic correlations. Despite these strong correlations, we can still use neural activity to decode task variables such as action selection and stimulus side. The performance of this decoding varies based on which brain areas the model uses. Remarkably, the performance of the decoder nearly matches the behavioral performance when using neural activity in V1, M2, and to a lesser extent, M1, however, the activity in other areas is less predictive of behavior despite strong global correlations. Current work now focuses on the effect of the prior on neural activity in different areas. The ability to probe multiple brain areas simultaneously provides a powerful approach to understanding how multiple brain areas coordinate to represent and solve decision making tasks.

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Poster

P498. Large Scale Analysis of Anatomy and Population Dynamics

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Program #/Poster #: P498.03

Topic: H.03. Decision Making

Support: Wellcome Trust (209558)

Wellcome Trust (216324)
Simons Foundation

Title: Neural population dynamics for hierarchical inference in mice performing the International Brain Lab task

Authors: *R. SCHAEFFER[1], N. BONACCHI[2], G. A. CHAPUIS[4], M. FAULKNER[5], J. M. HUNTENBURG[7], A. KHANAL[8], G. T. MEIJER[9], N. J. MISKA[10], A. PAN-VAZQUEZ[11], C. ROSSANT[5], M. SCHARTNER[3], K. Z. SOCHA[5], A. URAI[13], M. J. WELLS[5], O. WINTER[14], S. J. WEST[6], A. K. CHURCHLAND[15], I. B. WITTEN[12], I. R. FIETE[1], I. BRAIN LABORATORY[5];
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Abstract: We study the neural basis of probabilistic binary decision making in mice performing the International Brain Lab task, which combines perception of noisy stimuli with tracking a slowly shifting bias in the probability that one of the two possible actions is correct. Performing well at this task requires performing hierarchical inference over disparate timescales separated over 1-2 orders of magnitude. We generate hypotheses for how the brain might solve the task by training a continuous-time recurrent neural network. The model makes four key predictions we seek to test: First, the encoding of two key latent variables, stimulus side and bias side, is non-orthogonal, enabling interaction between the latent variables in a way that subserves hierarchical inference. Second, within-trial sensory evidence moves the neural state in the appropriate direction along both dimensions, albeit with different amplitudes that is related to the difference in timescales between the latent variables. Third, feedback from the previous trial, which in principle should enable more accurate inference of the bias, should have no effect on the integration of information. Fourth, that movement in the two-dimensional plane should display two kinds of dynamical behavior: within trials, sensory evidence drives the neural state to one of two discrete fixed points to infer one latent variable, whereas between trials, the neural state relaxes to a line attractor to infer the other latent variable. We test these predictions by first analyzing behavior from 342 sessions, quantifying performance metrics and showing a wide distribution of performance across mice. Next, we perform preliminary analyses of brain-wide Neuropixels recordings from 3 high-performing mice, and find they match these four predictions, with both model and mice exhibiting similar sub-optimalities relative to optimal Bayesian agents. Our findings shed light on the neural mechanisms involved in hierarchical probabilistic inference.

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Poster

P498. Large Scale Analysis of Anatomy and Population Dynamics

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Program #/Poster #: P498.04

Topic: H.03. Decision Making

Support: Wellcome Trust (209558)
Wellcome Trust (216324)
Simons Foundation

Title: Coding of vision, action initiation, action selection, and expectation in single neurons across the brain in The International Brain Lab dataset

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Abstract: Vision, action selection, action initiation, and prior expectation arise from neuronal activity that may be distributed across brain regions. Here we delineate the spatial distribution of single-cell neuronal activity that is relevant for these processes. To achieve this, we introduce a dataset of brain-wide Neuropixels recordings from mice performing a visual discrimination task in which they turn a wheel left or right to indicate the location of a presumed visual stimulus. In each recording session, the probability of stimuli appearing on the left vs. right is 80% (or 20%) in blocks of consecutive trials. As of July 2021, the recordings consist of 756 penetrations across the mouse brain, covering 278 brain regions. To visualize the neural activity across the brain, we computed the peristimulus time histograms of average neural activity in each brain region for specific stimulus conditions. To quantify the sensitivity of single neurons to the behavioral task (baseline vs. task), visual stimulus (left vs. right location), action selection (left vs. right direction of turning), action initiation and prior expectation (left vs. right block-side), for each of these conditions, we computed the sensitivity metric of given condition based on the condition-combined Mann-Whitney U statistic. Specifically, we compared the firing rates from each trial of one condition value to each of the other values, while fixing the other conditions, and then combined all comparisons into one number per neuron. In this way, we could isolate the influence of individual conditions on neural activity. To estimate statistical significance, we used a shuffle test in which trial labels were randomly permuted within the condition 3000 times. To control for spurious correlations due to serial dependencies in the task variables, we also used the

pseudo-session approach for the block-side, visual stimulus and action selection. We then computed the fraction of neurons in each brain region significant for behavioral task, visual stimulus, action selection, action initiation and prior expectation, and identified brain regions that are most relevant for these conditions. To further study the encoding of visual information of single neurons across the brain, we analyzed the receptive fields of neurons that are sensitive to visual stimuli and compared these responses to visual responses during the task. We also compared the activity during the task to activity while the same stimuli are presented in a passive-viewing paradigm. Consistent with prior work, we observed widespread coding of action initiation but substantially more restricted representations of visual and choice coding.

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Poster

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Topic: H.03. Decision Making

Support: Wellcome Trust (209558)
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Simons Foundation

Title: Motor correlates of cognitive variables in the international brain laboratory task

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Abstract: In the IBL task, a head-fixed mouse is trained to move a wheel to the left or right, depending on the side of a Gabor patch shown on a screen in front of the animal. The probability of a stimulus being on one side is 80% (20%) in blocks of consecutive trials before switching in an unsignalled manner. The animals' action selection (including on trials with 0% contrast input which are directly revealing of any bias) indicates that they are sensitive to this preponderance. We are currently examining neural correlates of this partly persistent sensitivity.
Here we explore aspects of the pre-stimulus behavior of the subjects to see if information about

the currently favored side (crudely a 'prior') is present in such a way that might confound or at least complicate our neural analysis. We investigated kinematic correlates, such as body posture or paw position on the wheel, and dynamic correlates such as laterally-dependent whisking, tongue movements/licking, or motion of the wheel. To do this, we had to control for the substantial autocorrelation in the favored side (and the aspects of this prior to which the animals' behavior shows to be sensitive).
We found that in a measurable fraction of animals, whisker movement between trials is modulated by block type, meaning that there is for example more whisking when a right stimulus is more likely to appear. Here we report the prevalence of such kinematic and dynamic correlates of the prior.

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Poster

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Support: Wellcome Trust (209558)
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Simons Foundation

Title: Task-relevant population dynamics in the international brain lab dataset revealed by manifold analysis

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Abstract: How animals use prior beliefs about their environment to make perceptual decisions is an important question in neuroscience. We analyze data from the IBL consisting of 583 Neuropixel recordings from mice performing a binary perceptual discrimination task (stimulus on left or right) in which the stimulus side probability varies across blocks of trials. Using unsupervised and supervised linear dimensionality reduction methods, we characterize the low-dimensional subspaces in which neural activity is confined, identify subspaces encoding key task-relevant variables, and analyze their within-trial population dynamics. To obtain task-

relevant subspaces we use a combination of two approaches. In the first, we compute averaged PSTHs by partitioning trials based on task variables, then apply principal component analysis to obtain a low-dimensional projection of task-relevant neural activity. We use a pseudo-session approach to assess the significance of distances between trajectories. This approach identifies sessions in which post-stimulus, pre-action trajectories for left and right choices are significantly separated, revealing the temporal evolution of stimulus integration and choice selection within the population. We also found a significant separation of trajectories prior to stimulus onset, possibly reflecting an internal bias based on the block. Our second approach uses linear discriminant analysis to obtain low-dimensional subspaces in which each axis discriminates a distinct task variable. We restrict these analyses to individual brain regions and identify regions in which there is significant discrimination of block side and action selection. We then project neural activity onto the subspace spanned by the discriminants for these variables. By identifying regions with significant discrimination of both block side and action selection, we consider how these two variables covary by analyzing within-trial trajectories in this subspace. This analysis reveals an effect of block in the action discriminant prior to stimulus onset and an effect of action selection on the block discriminant following stimulus onset. We find qualitatively similar dynamics across several brain regions.

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Poster

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Title: Dissecting Motor Representations by Video Analysis Using Deep Learning

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Abstract: Neural representations in cognitive tasks are typically considered in terms of abstract cognitive performance, yet it is known that cognitive and emotional states can be associated with typical movements, e.g., pacing when nervous, creating difficulty in the assignment of neural representation to the internal state or the movement. Here we analyze simultaneous neural recordings across multiple brain areas and highspeed videos of behavior in a short-term memory (STM) task (see Susu Chen et al SFN 2021). We test a series of approaches to relate neural activity to ongoing movements, from markers of facial features, through convolutional auto-encoder applied to videos to full end-to-end learning.
We found that areas more associated with motor control (e.g., the medulla for facial movements), had higher single-trial explained variance than areas related to motor preparation (e.g., the Anterior Lateral Motor cortex, ALM, part of M2). We find that more expressive models more accurately predicted neural activity. Auto-encoder embeddings were more accurate than facial markers, and end-to-end learning more accurate than embeddings.
We varied the time offsets between the behavior used for prediction and neural activity and found that offsets vary across brain regions and within layers in brain regions. We found that shallower layers in ALM were more associated with sensory activity than deeper layers, consistent with previous circuit mapping findings from Hooks et al. J. Neuroscience 2013. and Economo et al. Nature 2018. We tested the relation between trial-type selectivity of neurons and their predictability from video. We find that neurons that are more associated with trial-type rather than upcoming choice were harder to predict from videos, consistent with a more sensory representation. We demonstrate that the structure of neuronal correlations changes across subspaces of activity that are well or poorly predicted by behavioral representations.
We directly predicted upcoming choice from behavior representations. We find that upcoming choice was predictable, but accuracy was highly variable across animals. By examining the accuracy of different behavioral features we find that the movements predictive of future actions varied across animals.
These results shed light on the relation between movements and neural activity in STM tasks and demonstrate important steps in the effort to link neural representations to movement.

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Poster

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Title: Comparing cognitive models of dynamic reward learning in the head-fixed mouse

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Abstract: Comparing cognitive models of dynamic reward learning in the head-fixed mouse Jin Oh, Kevin J Miller*, Anna Lebedeva, Kenneth D Harris*
To characterize the behavioral strategies of reward-based learning, we quantitatively fit multiple cognitive models to behavioral data from head-fixed mice performing a two-armed bandit task. In this task, the start of each trial is signaled by an auditory cue, after which the mouse turns a wheel to the left or right side to earn a liquid reward. The mouse is rewarded with a 80% probability for choosing the favored side, and 20% probability for the unfavored side. The favored side flips randomly after 125 -175 trials.
We considered six different cognitive models, each representing a possible strategy mice might use to solve the task. The models were standard Q-learning (Sutton and Barto, 1998), optimistic Q-learning (Lefebvre et al., Nat. Hum, 2017), generalized local matching law (Baum, J. Exp. Anal. Behav.,1974), forgetting Q-learning (Barraclough et al., Nat. Neurosci, 2004), differential forgetting Q-learning (Ito et al., J Neurosci, 2009), and a mixture-of-agents model (Miller et al., bioRxiv, 2021). We assessed model fit in two different ways. First, to characterize which specific features of the data each of the models gets right, we created synthetic behavioral datasets using each model with parameters optimized separately for each mouse. We compared these synthetic datasets to the true behavioral dataset using multiple summary statistics, including trial-history regression (Lau et al., J Exp Anal Behav, 2005), bout distribution, and learning curves. Second, to obtain a single numerical score quantifying each model's fit, we used cross-validation: the model parameters were fit by maximum likelihood on one half of the data and evaluated by their likelihood on the other half. Based on these assessments, we found that the differential forgetting Q-learning and a mixture-of-agents model fit the mouse datasets better than the other cognitive models that we considered. We are currently exploring several additional models. Future work correlating the internal variables of these models with neuronal population recordings may help uncover the brain mechanisms of reward-based learning.
Wellcome Trust 205093/Z/16/ZKwanjeong Educational Foundation
2121/2300 characters

Disclosures: J. Oh: None. **K.J. Miller:** A. Employment/Salary (full or part-time):; Google Deepmind. **A. Lebedeva:** None. **K.D. Harris:** A. Employment/Salary (full or part-time):; University College London.

Poster

P498. Large Scale Analysis of Anatomy and Population Dynamics

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P498.09

Topic: H.03. Decision Making

Support: Gatsby Charitable Foundation (GAT3755)
Wellcome Trust 205093/Z/16/Z
Wellcome Trust 204915/Z/16/Z
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Wellcome Trust (219627/Z/19/Z)

Title: Recording neurons across the brain during a two-armed bandit task

Authors: *A. LEBEDEVA, K. J. MILLER, J. OH, Y. WANG, K. D. HARRIS;
Univ. Col. London, London, United Kingdom

Abstract: Learning from rewards in order to optimize behavior in a changing world is a key component of cognitive flexibility. In animals, this capacity is often studied using the "dynamic two-armed bandit" task. In each trial of this task the animal selects one of two possible actions, each of which is associated with a different time-varying probability of reward. A large body of work has sought to understand the neural mechanisms of reward learning using tasks of this kind, but studies have typically focused on a relatively small number of brain regions.
Here, we developed a version of the two-armed bandit task for head-fixed mice. The start of each trial was signalled by an auditory cue, after which the mouse chose to spin a steering wheel either to the left or to the right. On each trial, one of these (left or right) was designated the better choice, and followed by liquid reward with high probability, while the other was followed by reward only with low probability. The identity of the better choice flipped periodically throughout each behavioural session, requiring mice to continually learn in order to optimize the rate of rewards. Mice did this successfully, altering their choice on average within ~10 trials after the flip.
We used high-density Neuropixels probes to make acute extracellular recordings from ~21000 neurons across 15 brain regions (such as primary and secondary motor cortices, prelimbic and infralimbic cortex, caudoputamen, hippocampus, nucleus accumbens and others). Analysis of the data revealed that choice and reward coding was widespread, but that an interaction between choice and reward was most strongly encoded in the secondary motor cortex (MOs). This suggests that MOs plays a special role in reward-guided learning. To investigate this region in more detail, we implanted probes chronically, allowing us to record the activity of the same MOs neurons each day for multiple weeks, while animals were learning the task and as they performed the task after learning. In this dataset as well, we found a large number of MOs neurons encoding the interaction between reward and choice. Currently, we are developing reinforcement learning models of mouse behaviour in this task, and using them to search for correlates of cognitive variables such as correlates of expected reward. We are also investigating the role of the secondary motor cortex using causal methods, using optogenetic silencing on a subset of trials to determine whether neural activity in this region is necessary for trial-by-trial reward learning.

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Poster

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Title: Intrinsic timescales as an organizational principle of neural processing across the whole rhesus macaque brain

Authors: *A. M. G. MANEA[1], A. ZILVERSTAND[2], S. R. HEILBRONNER[1], J. ZIMMERMANN[1];
[1]Dept. of Neurosci., Univ. of Minnesota, Twin Cities, Minneapolis, MN; [2]Psychiatry, Univ. of Minnesota, Minneapolis, MN

Abstract: Intrinsic timescales (ITs) are rhythmic dynamics of neuronal fluctuations that serve to integrate and support neural processing on different timescales. Thought to reflect the amount of temporal integration of a brain area, they are closely related to cortical and subcortical functional hierarchy. Traditionally, they have been investigated using electrophysiology in nonhuman primates (Murray et al., 2014; Cavanagh et al. 2016; Cirillo et al., 2018; Fascianelli et al., 2019; Nougaret et al., 2021). However, this method is not suitable for understanding whole brain dynamics, nor is it straightforward to apply it in humans. Functional magnetic resonance imaging (fMRI) could solve these issues, since it allows for whole brain inquiries in both species. It has not been clear up until this point whether ITs can be assessed using fMRI. Because prior IT work has been done in nonhuman primates, here we assess whether previously identified IT hierarchies can be extracted from macaque resting-state fMRI. We analyzed ultra-high field (10.5T) resting-state fMRI from macaques (N=9). ITs were computed by estimating the autocorrelation function of each voxel's fMRI signal. We tested the fMRI replication of electrophysiological ITs in 18 regions from previously published studies. In nearly all cases, hierarchical IT patterns were replicated in fMRI. For example, sensory areas had the lowest and prefrontal cortex (PFC) the highest ITs. Because of this, we took the opportunity to interrogate ITs across the whole brain. We then compared IT maps with fine-grained resting-state functional connectivity (FC) gradients, which provide a mapping of FC similarity profiles. Since gradients interrogate FC similarity profiles across the brain, and hence its functional architecture, linking

them to the IT hierarchy can demonstrate that the underlying functional architecture of resting-state FC is not only based on low frequency synchrony, as is widely accepted, but rather reflects a more intricate “timescale hierarchy”. To establish this, gradients were estimated by applying diffusion mapping to the voxel-wise FC matrix of the area under investigation. We then established a relation between FC gradients and ITs by correlating both maps. For example, among others, ITs and gradient maps were highly correlated in the parietal lobe ($r = 0.56$), medial PFC ($r = 0.70$), and motor cortex ($r = 0.68$). We therefore propose that ITs underlie the functional architecture of resting-state FC, as measured by FC gradients, and that ITs describe the macroscale functional organization of the brain, both in terms of connectivity and intrinsic temporal fluctuations.

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Poster

P498. Large Scale Analysis of Anatomy and Population Dynamics

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Program #/Poster #: P498.11

Topic: D.08. Visual Sensory-motor Processing

Support: European Commission Grant 795846
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Wellcome Trust Grant 108726

Title: Representation of task variables in mouse frontal cortex during delayed decisions based on vision and value

Authors: *L. E. WOOL[1], E. SVAHN[1], R. TERRY[1], A. RITOUX[1], A. LAK[2], M. CARANDINI[1], K. D. HARRIS[1];
[1]Univ. Col. London, London, United Kingdom; [2]Oxford Univ., Oxford, United Kingdom

Abstract: [Introduction] During visually guided decision-making, the brain must optimally combine sensory evidence, reward value, and action timing to make the appropriate choice. However, it is not clear how, and where, these task variables are combined. In the mouse, frontal cortical area MOs is an important region for sensory-based decision-making (Coen et al., 2021; Zatka-Haas et al., 2021) and may encode upcoming choice in delayed-response tasks (Guo et al., 2014). [Methods] We asked whether MOs combines neural representations of task variables. To investigate this, we trained mice in a visually guided instructed delay task. Head-fixed mice report the location of a visual grating of variable contrast by steering a wheel left or right (Burgess et al., 2017) after an enforced delay period of 0.8-1.0 s. The volume of water rewarded

for correct choices differed twofold between correct left and correct right choices, and alternated over blocks of ~200 trials (Lak et al., 2020). We used two-photon calcium imaging to measure the activity of thousands of L2/3 cortical excitatory neurons in MOs while mice performed the task. [Results] Performance on a given trial depended on both stimulus contrast and expected reward. Psychometric curves shifted between blocks, favoring choices promising larger rewards. Furthermore, the proportion of impulsive choices (reports made before the end of the delay) increased with expected reward and stimulus contrast. MOs neurons heterogeneously coded multiple task variables. A minority of neurons exhibited contralateral visual responses at the time of stimulus onset. Most neurons showed nonselective modulation at movement onset, but many were also choice-direction-selective. Neurons with significant contraversive or ipsiversive choice preference were found in both MOs hemispheres. Some neurons were modulated by trial outcome in the feedback period, encoding the presence or absence of reward and/or licking activity. Other neurons were differentially modulated by whether the animal delayed their choices or moved impulsively. Yet other neurons appeared to be modulated by task-independent variables such as whisking. Some MOs neurons exhibited mixed selectivity to multiple task variables. [Conclusions] Our preliminary findings suggest that excitatory MOs neurons heterogeneously encode features of a visually guided choice delay task, as well as task-independent features such as whisking. Moreover, some MOs neurons encode multiple features, suggesting a role in combining representations of task variables during decision-making.

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Poster

P501. Cross-Modal Processing II

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Program #/Poster #: P501.01

Topic: D.09. Multisensory Integration

Support: NIH Grant 1R01NS118461-01

Title: Behavioral states in a context-dependent multi-modal sensory task

Authors: *D. HULSEY[1], K. ZUMWALT[1], M. SMEAR[2], L. MAZZUCATO[3], D. A. MCCORMICK[1], S. JARAMILLO[4];
[1]Inst. of Neurosci., [2]Inst. of Neuroscience, Dept. of Psychology, [3]Insitute of Neuroscience, Departments of Biol. and Mathematics, [4]Inst. of Neuroscience, Dept. of Biol., Univ. of Oregon, Eugene, OR

Abstract: During natural behavior, contingencies between stimuli and outcomes often depend on context, and animals must flexibly adjust their behavioral strategies accordingly. Changes in

brain state are known to influence performance during fixed-contingency sensory tasks, and we hypothesize that brain state dynamics underlie an animal's ability to behave in a flexible, context-appropriate manner. To capture context-dependent brain state dynamics, we mapped behavioral states during a paradigm where mice must flexibly adjust their strategy in a context dependent manner. In our paradigm, head-fixed mice discriminate between frequencies of tone clouds or angles of drifting gabor patches, while we measure pupil size, whisker pad motion, and locomotion. We analyzed task performance using a Hidden Markov Model with states parameterized by a Generalized Linear Model (GLM-HMM). The GLM-HMM revealed that each mouse used multiple strategies throughout task performance, and the model identified transitions between the distinct decision-making states on a trial by trial basis. We used this framework to interrogate the relationship between decision-making strategies and measured behavioral variables. In parallel with developing our behavioral paradigm, we have implemented high density electrophysiological recordings and single-cell resolution neural imaging across broad regions of the temporal and dorsal cortical surface. Combining these recording techniques with our state-based description of task performance will enable us to observe context-dependent changes in neural response dynamics across cortical regions. These efforts will build an understanding of the brain states that underlie behavioral flexibility.

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Poster

P501. Cross-Modal Processing II

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Program #/Poster #: P501.02

Topic: D.09. Multisensory Integration

Support: SWC PhD programme (The Gatsby Charitable Foundation GAT3361 and The Wellcome Trust 090843/F/09/Z)
Wellcome Trust Grant 205093

Title: Additive audiovisual integration in the mouse superior colliculus

Authors: *F. TAKACS[1], P. COEN[2], M. ROBACHA[2], K. D. HARRIS[2], M. CARANDINI[2]; [1]Sainsbury Wellcome Ctr., [2]Univ. Col. London, London, United Kingdom

Abstract: [Introduction] To guide actions, the brain usually integrates information from multiple senses. For instance, an object can be located more accurately by combining visual and auditory cues to its position. In multiple species, neural correlates of spatially selective audiovisual integration have been found in the superior colliculus (SC, Stein & Meredith, Science, 1983, Knudsen,J.Neurosci,1982, King et al., J. Comp. Neur., 1985). How prevalent are audiovisual

neurons in the SC of the mouse? Are they spatially selective for auditory stimuli? Do they integrate audiovisual signals nonlinearly (Ito et al., bioRxiv, 2021) or additively as has been seen in the frontal cortex (Coen et al., bioRxiv, 2021)? [Methods] To address these questions, we recorded the activity of neural populations (~2,400 neurons) in SC of awake, head-fixed mice using multishank Neuropixels 2.0 probes, in response to checkerboard visual stimuli and pink noise auditory stimuli presented at varying azimuths, alone or in combination. [Results] We found neurons selective for visual stimuli (26% of neurons) mostly in the superficial layers, and neurons selective for auditory stimuli (20%) mostly in intermediate and deep layers. 8% of neurons responded to both stimuli. While essentially all visual neurons were spatially tuned following a topographic visual spatial map, only 21% of auditory neurons were spatially tuned, and the tuning of these auditory neurons did not form a spatial map. We found that 95% of neural responses were captured by an additive model. The remaining neurons tended to be visual rather than multisensory, with visual responses modulated nonlinearly by the presence of sounds. [Conclusions] Overall, these results suggest that the SC, like the frontal cortex, combines signals from the different senses additively. This strategy is optimal under broad statistical assumptions. We are currently testing whether this additive combination holds only during passive stimulus presentation or also during audiovisual behavior (Coen et al., bioRxiv, 2021).

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Poster

P501. Cross-Modal Processing II

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Topic: D.09. Multisensory Integration

Support: Wellcome Trust Grant 531073
Wellcome Trust Grant 205093
Wellcome Trust Grant 204915
ERC Grant 694401
ERC Grant 531949

Title: Mouse frontal cortex mediates additive multisensory decisions

Authors: *P. COEN[1], T. P. H. SIT[2], M. J. WELLS[1], M. CARANDINI[1], K. D. HARRIS[1]; [1]Univ. Col. London, London, United Kingdom; [2]Sainsbury-Wellcome Ctr., London, United Kingdom

Abstract: [Introduction] To interpret the world and make accurate perceptual decisions, the brain must combine information across sensory modalities. For instance, it must combine vision

and hearing to localize objects based on their image and sound. Probability theory suggests that evidence from multiple independent cues should be combined additively, but it is unclear whether mice and other mammals do this, and the cortical substrates of multisensory integration are uncertain. Here we show that to localize a stimulus, mice combine auditory and visual spatial cues additively, a computation supported by unisensory processing in auditory and visual cortex and additive multisensory integration in frontal cortex. [Methods] We developed an audiovisual localization task where mice turn a wheel to indicate the joint position of an image and a sound. The stimuli can be auditory, visual, or a combination of the two, presented in coherent or conflicting locations. In a set of mice, we performed optogenetic inactivation of different spots across dorsal cortex while mice performed this task. We then focused our recordings on frontal cortex, recording >10,000 neurons with Neuropixels probes during behaviour. [Results] In this task, mice integrated auditory and visual cues independently in all conditions. An additive model, with independent weights for audio and visual stimuli, predicted the mean response of mice to all cue combinations. Optogenetic inactivation of different spots across dorsal cortex while mice performed this task demonstrated that auditory and visual areas contribute unisensory information, whereas frontal cortex (secondary motor area, MOs) contributes multisensory information to the mouse's decision. Of the frontal areas recorded, only MOs activity could predict the mouse's upcoming choice. Consistent with the mice's additive behavior, neural activity in MOs reflected an additive combination of visual and auditory signals. Furthermore, an accumulator model applied to the sensory representations of MOs neurons reproduced behaviourally observed choices and reaction times. [Conclusions] Frontal area MOs integrates information from multiple sensory cortices additively, providing a signal that is then transformed into a binary decision by a downstream accumulator.

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Poster

P501. Cross-Modal Processing II

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Program #/Poster #: P501.04

Topic: D.09. Multisensory Integration

Support: Wellcome Trust Grant 205093

Title: Additive multisensory integration across species and modalities

Authors: *M. CARANDINI, T. SIT, K. D. HARRIS, P. COEN;
Univ. Col. London, London, United Kingdom

Abstract: [Introduction] To make accurate perceptual decisions, the brain must combine signals from different sensory modalities. A simple way to combine these signals is to add them. This procedure is optimal if the signals are statistically independent and may be a useful heuristic if they are not. We recently found that mice use this heuristic when combining sounds and images to localize a target (Coen et al, *bioRxiv* 2021). Here we ask whether this heuristic could also describe multisensory integration in different modalities and species. [Methods] We analyzed published data from studies where rats, macaques, and humans perform a two-alternative discrimination task that encourages the combination of two sensory modalities, and we used the "GRABIT" function in Matlab to estimate the values from the published psychometric data. These studies involved (1) a task where macaques used visual and vestibular cues to determine the direction of heading (Gu et al, *Nature Neurosci*, 2008); (2) a task where rats and humans used visual and auditory cues to estimate stimulus rate (Raposo *et al, J. Neurosci*, 2012, and Pisupati et al, *eLife*, 2021); (3) a task where rats combined visual and tactile cues to determine the orientation of a grating (Nikbakht et al, *Neuron*, 2018). [Results] In all these studies, the psychophysical data appeared to be well fit by a simple additive model in which the log odds of choosing one option vs. the other are the sum of the log odds estimated from one sensory modality and the log odds estimated by the other sensory modality. This was particularly clear when replotting the psychometric curves in log odds scale: in this representation, the responses to stimuli in each modality fall on a straight line and summing those two lines yields the responses to stimuli involving both modalities. [Conclusions] These results resemble those we obtained in mice that used visual and auditory cues to determine the location of a stimulus (Coen et al, *biorXiv*, 2021). Addition of sensory signals from different modalities, therefore, seems to be a heuristic that can quantitatively explain the way multisensory evidence is combined across multiple species and behavioral tasks. It may thus represent a default mechanism by which the brain integrates signals across modalities.

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Poster

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Topic: D.09. Multisensory Integration

Support: Sainsbury Wellcome Centre PhD program
Wellcome Trust Grant 205093
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Title: Behavioral responses to auditory and visual stimuli in mice

Authors: *T. P. H. SIT[1], C. BIMBARD[2], M. ROBACHA[2], P. COEN[2], M. CARANDINI[2], K. D. HARRIS[2];
[1]Sainsbury-Wellcome Centre, Univ. Col. London, London, United Kingdom; [2]Univ. Col. London, London, United Kingdom

Abstract: [Introduction] Multisensory responses are apparently found throughout the brain, even in areas that were thought to be devoted to particular sensory modalities. However, assigning this neural activity to sensory variables per se is confounded by the fact that much of the brain exhibits activity related to movement, which can be evoked by sensory stimuli. Based on results in (Bimbard et al 2021, BioRxiv), we hypothesize that many of the stimuli commonly used in sensory research evoke movement responses that were not robustly characterized. Here we sought to characterize the diversity of movement found in mice in response to sensory stimuli commonly used in audio and visual neuroscience.
[Methods] We presented head-fixed mice with a large set of auditory, visual and audiovisual stimuli. Stimuli were drawn from studies in sensory and multisensory neuroscience, and include drifting gratings, moving bars, looming discs, pure tones, chirps, ultrasonic recordings of mice, and naturalistic movies. At the same time, we filmed mice with three cameras capturing the whiskers, eye, paws, and torso and monitored their breathing with flow sensors.
[Results] Sounds such as pure tones consistently triggered an onset movement response. This response persisted both within an experiment across the span of hours and between experiments across weeks. Visual looming discs elicited movement responses that are more pronounced at a specific range of looming speed, and there was an asymmetry in elicited movement between white looming discs presented with a black background and black looming discs presented with a white background. Naturalistic sounds elicited complex movement responses that could not be easily predicted from their spectrotemporal properties. These more complex relationships varied across mice.
[Conclusion] We conclude that auditory and visual stimuli commonly used in neuroscience research can evoke different magnitudes and types of movement in mice, which can sometimes be hard to predict from basic properties of the stimuli. These movements persist after habituation to the stimuli, so they are likely to affect all recordings, even after habituation. These results highlight the importance of controlling for movement variables when studying the correlates of neural activity.

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Poster

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Topic: D.09. Multisensory Integration

Support: EMBO ALTF 740-2019
Wellcome Trust 205093

Title: Behavioral origin of sound-evoked activity in mouse visual cortex

Authors: *C. BIMBARD, T. P. H. SIT, A. LEBEDEVA, K. D. HARRIS, M. CARANDINI; Univ. Col. London, London, United Kingdom

Abstract: [Introduction] Many studies suggest that all of sensory cortex, including primary sensory areas, is multisensory. For instance, mouse primary visual cortex (V1) appears to be influenced by auditory signals. This influence may be due to projections from the auditory system to the visual cortex. However, there is a possible alternative explanation for these apparent multisensory signals. Sounds can change internal state and evoke uninstructed body movements. Internal state and body movements correlate with activity in many brain regions, including V1. It is thus possible that sounds affect visual cortex because they change internal state or behavior.
[Methods] To test this possibility, we asked whether sound-evoked signals in mouse V1 could be predicted by uninstructed sound-evoked movements. Using Neuropixels probes, we recorded the responses of hundreds of V1 neurons to audiovisual stimuli, while filming the mouse. We monitored internal state through pupillometry, as well as specific body and face movements.
[Results] We observed that V1 encoded a low-dimensional representation of sounds (different neurons showing similar responses), in contrast to the encoding of videos, which was high-dimensional (different neurons showing different responses). The time courses of the sound responses in this low-dimensional space were tightly correlated to the body movements evoked by these sounds. Different sounds evoked different temporal patterns of movement, which were stereotyped across trials and across mice, and these movements could predict the sound responses of V1 neurons.
[Conclusion] We conclude that the apparent representation of sounds in visual cortex can be explained by uninstructed yet stereotyped sound-evoked movements. This result suggests that the multisensory activity that has been widely observed across the brain may have a simpler, behavioral origin.

Disclosures: C. Bimbard: None. **T.P.H. Sit:** None. **A. Lebedeva:** None. **K.D. Harris:** None. **M. Carandini:** None.

Poster

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Topic: D.09. Multisensory Integration

Support: NIH Grant 1U01NS113252-01

Title: Spiral waves of activity in the cortex of awake mice

Authors: *Z. YE, N. A. STEINMETZ;
Biol. Structure, Univ. of Washington, Seattle, WA

Abstract: Travelling waves are often observed in large scale population activity in different brain areas and in various brain states. However, the detailed spatiotemporal patterns of wave propagation have not been well characterized in awake mammals. Using widefield imaging of the dorsal cortex in awake GCaMP7-expressing transgenic mice, we robustly observed spiral waves in the 2-8Hz frequency range. Spiral centers were detected using optical flow methods applied to phase data after Hilbert transformation. These spirals were usually mirrored on the left and right hemispheres with opposing rotation directions. Similarly, we frequently observed simultaneous mirrored spirals split along the S1/M1 border within the same hemisphere. Spirals tended not to occur during overt movements of the mouse, and were more prevalent during periods of quiet wakefulness, which are also characterized by high 2-8Hz power. Spirals were also present in epochs during which mice actively consumed rewards, though primarily in between moments of reward licking. The presence of spirals in wild-type mice was separately validated with 4-shank Neuropixels 2.0 probe recordings in the cortex. These results demonstrate the cortical activity in the mouse has more complex spatiotemporal patterns, including distinctive spirals, than previously known.

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Poster

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Topic: D.09. Multisensory Integration

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The Wellcome Trust 214333/Z/18/Z; 090843/F/09/Z

Title: Active translation signals underpin sensory-motor mismatch responses in the mouse primary visual cortex

Authors: M. VELEZ-FORT, L. COSSELL, ***T. MARGRIE**;
Sainsbury Wellcome Ctr., London, United Kingdom

Abstract: To accurately navigate, animals must integrate multiple types of information whilst locomoting - that is, moving from one place to another. Importantly, motor commands must be constantly compared with ongoing feedback about the actual motion of the head (and eyes) as well as external visual motion. The primary visual cortex in mice receives both motor and vestibular input that is presumably combined with visual information during locomotion and navigation. Here, we have for the first time investigated neural activity in V1 recorded during locomotion in its most basic form: running forward in a single dimension.
Using a novel setup in head-fixed mice that allows us to independently manipulate the 3 fundamental elements of locomotion - motor/running (R), translation (T) and visual flow (VF) - we found that 74% of V1 neurons increase their activity during locomotion (i.e., when the speed of running, translation and visual flow are matched) compared to standing still. To understand the contribution of translation to overall locomotion activity, we first compared neural responses while animals were not permitted to run (treadmill locked), but experienced visual flow (VF) or passive translation with visual flow (T+VF). We found that in 59.5% of all recorded V1 cells, firing rates during T+VF motion were significantly greater than firing rates during VF alone, suggesting the existence of widespread translation-based input to V1. However, the modulation of activity due to translation was strongly attenuated during active locomotion. Only 6% of V1 cells showed any difference in motion-evoked activity when comparing running with visual flow (R+VF) to active locomotion (R+T+VF). This suggests that although V1 has access to translation signals, during locomotion, translation-based activity in V1 is largely attenuated.
To explore the functional role that these translation inputs may play, we randomly introduced brief (250ms) mismatch events by increasing the gain in both translation (T) and visual flow (VF) while the animal was engaged in active locomotion. These ‘slip’ events triggered strong responses across V1. At a single cell level, 20% of cells showed a significant change in firing rate. These responses couldn’t be explained by the mismatch between running speed (R) and visual flow (VF) alone, since omitting translation led to a complete absence of mismatch-related firing rate changes.
Taken together, these results suggest a circuit motif that enables attenuation of translation-based signals by motor input that serves as the substrate for a locomotion-related mismatch signalling and that V1 is acutely sensitive to un-expected movement of the head.

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Poster

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Support: RTG2416 MultiSenses - MultiScales

Title: Multisensory integration in superior colliculus and primary visual cortex of awake behaving mice

Authors: *K. DOERENKAMP[1,2], P. S. GRAFF[3,2], G. NABBEFELD[1,2], T. RÜLAND[4,1,2], S. BRILL[1], B. M. KAMPA[1], S. MUSALL[3];
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Abstract: The Superior Colliculus (SC) is a key area for the integration of multisensory stimuli and involved in numerous cognitive functions. Inputs from different modalities reach the SC either directly or via the cortex. Yet, how multisensory integration in SC differs from cortex is not well understood. We therefore performed Neuropixels recordings in visual cortex (V1) and SC of awake mice while presenting visual, auditory and tactile stimuli. A majority of V1 neurons responded to visual stimuli but a large fraction also responded to auditory and tactile stimulation. In SC, responses were more evenly distributed with a small preference for visual and tactile over auditory stimuli.
To quantify multisensory integration in V1 and SC neurons, we compared responses to unisensory stimuli to different multisensory combinations. Here, multisensory were mostly larger than unisensory responses for all stimulus combinations. Interestingly, multisensory enhancement was more common in V1 while SC neurons showed a larger variety in either enhancement or depression, suggesting a more detailed representation of multisensory stimuli. Accordingly, SC neurons were also more sensitive to spatially incongruent multisensory stimuli which promoted multisensory depression in SC but not in V1.
To assess the role of cortical inputs for responses in SC, we optogenetically stimulated either V1 or primary somatosensory cortex (S1). Excitation of V1 or S1 reliably activated SC neurons with short latencies, confirming that they were driven by direct cortical projections. Optogenetic inhibition of V1 or S1 strongly reduced corresponding sensory responses in the SC, suggesting that cortex is the primary source for sensory responses in SC. S1 stimulation also drove V1 neurons but with significantly longer latencies, suggesting that tactile responses in V1 are due to indirect S1 inputs. Lesions in the rostrolateral visual area RL strongly reduced all tactile responses in V1.
Lastly we tested how multisensory integration is affected by behavior. We thus trained mice in a multisensory discrimination task where visual gratings and tactile stimuli had to be integrated to obtain a reward. Mice performed the task with high accuracy and we compared multisensory responses in V1 and SC in naive versus trained animals. Here, multisensory responsiveness was stronger in trained mice.
Our results demonstrate that multisensory integration in the SC largely depends on cortical inputs but is more sensitive to incongruent stimuli. This and its increased multisensory responsiveness emphasizes its importance for multisensory perception and behavior.

Disclosures: K. Doerenkamp: None. **P.S. Graff:** None. **G. Nabbefeld:** None. **T. Rüland:** None. **S. Brill:** None. **B.M. Kampa:** None. **S. Musall:** None.

Poster

P501. Cross-Modal Processing II

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P501.10

Topic: D.07. Vision

Support: Brain Initiative Grant 1R01NS118461-01
NIH Grant 5R35NS097287-05

Title: Pan-cortical state-dependent activity exhibits complex spatiotemporal structure during spontaneous and stimulus-evoked behavior

Authors: *E. D. VICKERS, D. G. WYRICK, P. PARKER, C. NIELL, L. MAZZUCATO, D. A. MCCORMICK;
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Abstract: The flow of neural activity across cortex during active sensory discrimination is constrained by task-specific cognitive demands. During spontaneous behavior, the brain is free to sample widely from a vast repertoire of activation motifs. Understanding how local and global activity motifs are selected by task demands in a modality-dependent manner will require in-depth study of densely sampled spontaneous activity at single neuron resolution across large regions of cortex. Here we show 2-P Ca^{2+} imaging data from two novel *in vivo* preparations that enable mesoscale imaging of up to 7500 individual neurons simultaneously at ~3 Hz across a 6 x 6 mm FOV. In total, we imaged more than a half-million neurons over 200 sessions, each lasting ~45 min, across 20 mice. We recorded state variables of walk speed, pupil diameter, and whisker motion energy. Preliminary analyses revealed heterogeneous distributions of neurons with large correlations to behavioral state variables, and some neurons with no apparent correlation to arousal or movement. Exploration of the heterogeneity of state-dependence with partially supervised k-means clustering revealed diverse spatiotemporal patterns of activity (e.g. dense, widespread, and temporally structured) that were not evident with sorting by principal components, and had clear relationships to state variables (e.g. ON / OFF, transient / sustained). To test for directed causal interactions of putative long-range functional connections, we developed a novel statistical method called functional causal flow (FCF). Preliminary FCF analysis of widefield GCaMP6s imaging data from dorsal cortex revealed strong state-dependent modulation of inter-areal causal functional connectivity during passive visual stimulation relative to periods of darkness. We are currently performing similar FCF analysis of 2-P mesoscope data. In the future, we will explore how engagement, strategy and cognitive flexibility in a switching audiovisual 2-alternative forced choice (2-AFC) discrimination task act to further shape and select the subset of activity patterns present under spontaneous and passive conditions.

Disclosures: E.D. Vickers: None. **D.G. Wyrick:** None. **P. Parker:** None. **C. Niell:** None. **L. Mazzucato:** None. **D.A. McCormick:** None.

Poster

P596. Neuroendocrine Anatomy and Physiology

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P596.01

Topic: F.03. Neuroendocrine Processes

Support: Science for Life Laboratory

Title: Elucidating sex differences in the expression of aromatase in the brains of young adult rats by fluorescent in-situ hybridization

Authors: *J. IMMENSCHUH, S. B. THALHAMMER, E. COMASCO;
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Abstract: The enzyme aromatase (*Cyp19a1* gene) catalyzes the biosynthesis of estrogens from androgens. Of interest, aromatase seems to be involved in non-reproductive functions in the brain, such as regulation of neurogenesis, synaptic transmission and neuroprotection. Sparse evidence points to expression in specific brain regions that are of relevance to emotional and cognitive functions, and in a sex-dependent manner. Investigation of its expression throughout the brain has shown to be outdated and imprecise, and is lacking the identification of sub regions in both males and females. Additionally, little is known about the identity of the neurons expressing aromatase. The present study sought to confirm and map the expression pattern of *Cyp19a1* throughout the brain of young adult rats of both sexes and elucidate sex differences, as well as characterize in an exploratory fashion the cell types expressing this gene. Brains of male and female Sprague-Dawley rats (n=12) were collected on postnatal week ten and sliced coronally. *Cyp19a1* mRNA was mapped by use of fluorescent in-situ hybridization (FISH) and regions of interest were identified. Automated quantification of the number of positive cells per ROI was computed using CellProfiler. In the male rat brain, *Cyp19a1* was highly expressed in the posterior medial amygdaloid nucleus (MeP) and the bed nucleus of stria terminalis (BNST). Intermediate expression levels were found in the medial preoptic area (MPA), the Cornu Ammonis field 3 (CA3) of the hippocampus and the posterior cortical amygdalohippocampal area (AHi). Low expression was detected in the anterior caudate putamen (CPu), the nucleus accumbens (Acb) and the ventromedial hypothalamus (VMH). In the female rat brain, similar expression patterns were detected. However, the expression was significantly lower than in the males in the BNST, VMH, MeP, AHi and the CA3 field. Co-expression with cell markers was scrutinized with double FISH to examine the identity of *Cyp19a1* mRNA expressing cells. The majority of the *Cyp19a1*-positive cells were GABAergic, some also expressing somatostatin, in all regions except for the cortical amygdala and the anterior AHi where the highest co-expression

was found in glutamatergic cells. A smaller fraction of cells was shown to co-express *Slc1a3* especially in the amygdala and the VMH, suggesting expression of *Cyp19a1* also in astrocytes. No co-expression was observed with *Gfap, Slc18a2, Chat, Mbp, Th* or *Pvalb*. These findings suggest a sex-specific expression pattern of aromatase in the brain of young adult rats and that *Cyp19a1* is expressed region-specifically in several neural cell types.

Disclosures: J. Immenschuh: None. **S.B. Thalhammer:** None. **E. Comasco:** None.

Poster

P596. Neuroendocrine Anatomy and Physiology

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Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P596.02

Topic: F.03. Neuroendocrine Processes

Support: NIH-R15MH118692

Title: Sex differences in androgen receptor, estrogen receptor alpha, and c-Fos co-expression with corticotropin-releasing factor expressing neurons in restrained mice

Authors: *K. A. RYBKA, J. J. LAFRICAN, Z. J. ROSINGER, D. O. ARIYIBI, M. R. BROOKS, J. J. JACOBSKIND, D. G. ZULOAGA;
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Abstract: Prevalence rates of anxiety and depression are nearly twice as high in women when compared to men, with suggested mechanisms involving sex-dependent gonadal hormone exposure and hypothalamic-pituitary-adrenal (HPA) axis dysregulation. Actions of gonadal hormones through androgen receptor (AR) and estrogen receptor alpha (ERα) have been implicated in the regulation of HPA axis responsiveness and anxiety behaviors, while corticotropin-releasing factor (CRF) is known to regulate the HPA axis, anxiety, and depression. Using Crh-IRES-Cre::Ai9 (tdTomato) reporter mice we first tested whether CRF neurons co-localize AR in mice and if this differed by sex. High co-localization of AR in CRF neurons was found within the medial preoptic area (MPOA), bed nucleus of the stria terminalis (BST), medial amygdala (MeA), and ventromedial hypothalamus (VMH), while moderate CRF/AR co-expression was found within the central amygdala (CeA) and low levels in the paraventricular hypothalamus (PVN). Sex differences in CRF/AR co-expression were found in numerous brain regions including the BST, CeA, and VMH where males showed higher levels of co-expression within these regions. We next investigated CRF co-localization with ERα, where generally less CRF/ERα co-localization was found relative to CRF/AR co-localization. High co-expression was found within the MPOA and VMH, while moderate levels were found in the arcuate nucleus and low levels in the PVN and CeA. Sex differences in CRF/ERα were found in the MPOA and PVN, where females showed higher levels of co-localization. Finally, we restrained mice for 30

minutes to assess neural activation of CRF cells within these regions during psychogenic stress by assessing co-localization with the neural activation marker cFos. Greater CRF/cFos co-expression was found in females when compared to males in the principal nucleus of the BST, with no other sex differences found in other regions. Given the known role of CRF in the regulation of anxiety, depression, and the HPA axis, the presence of AR and ERα and sex-specific activation of discrete CRF cell groups indicate potential mechanisms for the mediation of sex differences in these functions.

Disclosures: K.A. Rybka: None. **J.J. Lafrican:** None. **Z.J. Rosinger:** None. **D.O. Ariyibi:** None. **M.R. Brooks:** None. **J.J. Jacobskind:** None. **D.G. Zuloaga:** None.

Poster

P596. Neuroendocrine Anatomy and Physiology

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Program #/Poster #: P596.03

Topic: F.03. Neuroendocrine Processes

Support: R01 MH086828
T32 GM008181-30

Title: Functional characterization of hippocampal regulation of CRF+ PVN neuronal activity

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[1]Physiol., Univ. of Maryland Sch. of Med., Baltimore, MD; [2]Univ. of Maryland Baltimore, Baltimore, MD

Abstract: Corticotropin-releasing factor-positive (CRF+) neurons in the paraventricular nucleus of the hypothalamus (PVN) drive activity of the hypothalamic-pituitary-adrenal (HPA) axis. The release of CRF from these cells initiates the neuroendocrine stress response that ultimately leads to elevation of circulating glucocorticoids and exerts effects on a wide range of body systems. This systemic and powerful response therefore demands tight regulation of the HPA axis, carried out by brain regions upstream of the PVN as well as negative feedback within hypothalamic, pituitary, and adrenal cells. Circulating glucocorticoid levels exhibit a complex and dynamic pattern of activity that respond to numerous physical and psychological stimuli, suggesting regulation by multiple brain regions. Although this negative feedback is crucial to the proper functioning of the HPA axis and ultimately an organism's health, certain psychiatric conditions have been linked to dysregulation of this negative feedback. One of the most consistent biological findings in patients with major depression is dysregulation of the HPA axis. Investigating links between these conditions and the dysregulation of the HPA axis may lead to important insights of the underlying biology of these conditions. The hippocampus is suggested as a prime candidate for contributing to regulation of HPA activity. Removing hippocampal

inputs to PVN leads to increased activity of the PVN, suggesting a net inhibitory influence. The exact nature of this regulation is unclear, and the complexity of HPA axis overall suggests that the answer may not be as straightforward as lesions studies suggest. The presented work utilizes patch clamp electrophysiology to record the activity of CRF+ PVN cells in response to electrical and optical stimulation of hippocampal inputs to the PVN. Results suggest that the hippocampus provides both monosynaptic excitatory and disynaptic inhibitory input to CRF+ neurons of the PVN, with the bed nucleus of the stria terminalis serving as an inhibitory relay for hippocampal excitatory output. Inhibitory postsynaptic potentials (eIPSCs) elicited with high frequency stimulation of hippocampal efferents maintained a fairly consistent amplitude in CRF+ cells, whereas excitatory postsynaptic potentials (eEPSCs) decreased in amplitude. The functional consequences of this input were studied in vivo, using optogenetic stimulation of hippocampal efferents and determination of plasma corticosterone levels. The BNST may thus represent a site of sign reversal, allowing glutamatergic hippocampal efferents to exert negative feedback on the HPA axis.

Disclosures: A.B. Cole: None. **S.M. Thompson:** None.

Poster

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Topic: F.03. Neuroendocrine Processes

Support: NIH Grant R01AG064091
P51 OD011133

Title: Changes in Cortisol Levels Across the Lifespan in Common Marmosets (Callithrix jacchus)

Authors: *M. LOPEZ[1,2], A. SEIDL[1], K. A. PHILLIPS[1,2];
[1]Neurosci., Trinity Univ., San Antonio, TX; [2]Southwest Natl. Primate Res. Ctr., Texas Biomed. Res. Inst., San Antonio, TX

Abstract: Human aging is associated with senescence of the hypothalamic-pituitary-adrenal axis, which leads to progressive dysregulation and increased cortisol exposure. This key hormone is implicated in the pathogenesis of many age-related diseases. Common marmosets display a wide spectrum of naturally-occurring age-related pathologies that compare similarly to humans, and are increasingly used as translational models of aging and age-related disease. Our previous work has shown cortisol concentration decreases in marmosets from juvenile to adult ages. Whether the marmoset hypothalamic-pituitary-adrenal axis also shows senescence with increasing age is unknown. To address this question, we analyzed hair cortisol concentration

(HCC) across the lifespan of captive common marmosets via a cross-sectional design. We collected shaved hair from the upper back from 48 marmosets from five developmental stages: infant (n=8), juvenile (n=10), adult (n=10), older adult (n=10), and geriatric (n=10). For all age classes an equal number of males and females were sampled. Samples were processed and analyzed for cortisol using enzyme immunoassay. HCC ranged from 1416 to 12408 pg/mg. We found significant main effects of age and sex (age: $F(4,38) = 7.74$, $p = .001$; sex: $F(1,38) = 7.00$, $p = .012$). Infants had significant higher levels of HCC compared to all other age groups. Females had higher HCC than males. The interaction between sex and age was not significant. These results indicate marmosets do not show dysregulation of the HPA axis as they age.

Disclosures: M. Lopez: A. Employment/Salary (full or part-time):; Trinity University. **A. Seidl:** None. **K.A. Phillips:** A. Employment/Salary (full or part-time):; Trinity University.

Poster

P596. Neuroendocrine Anatomy and Physiology

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Topic: F.03. Neuroendocrine Processes

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Brain & Behavior Research Foundation NARSAD Young Investigator Award
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Title: Neural circuitry for oxytocin release and maternal behavior

Authors: *S. VALTCHEVA[1], H. A. ISSA[1], K. A. MARTIN[1], K. JUNG[2], H.-B. KWON[2], R. C. FROEMKE[1];
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Abstract: Parenting behaviors emerge from complex neural circuits conferring sensitivity to infant needs to ensure survival of the species. One important molecular signal for the maternal brain is oxytocin (OT), a nine amino acid peptide produced mainly in the hypothalamus (Althammer 2017; Jurek 2018; Valtcheva 2019). Oxytocin is believed to powerfully enhance parental behaviors by increasing the salience of sensory cues from the offspring (Dölen 2015; Grinevich 2018; Froemke 2021). However, it remains unexplored what sensory cues from infants can activate OT neurons in new mothers. Here we describe a neural circuit routing auditory

information about infant vocalizations to the OT system in maternal mice (dams). By performing in vivo cell-attached and whole-cell recordings from optically-identified OT neurons, as well as fiber photometry of OT cells in awake dams, we found that OT neurons, but not other hypothalamic cells, are activated following playback of pup distress vocalizations. Using anatomical tracing and channelrhodopsin-assisted circuit mapping (Petreanu et al., 2007), we identified the projections and brain areas (from inferior colliculus and auditory cortex to the posterior intralaminar thalamus, PIL) relaying auditory information to OT neurons. We identify the PIL as a major auditory input to OT cells. In hypothalamic brain slices, we found that optogenetic activation of PIL fibers led to long-term depression of synaptic inhibition in OT neurons mediated by postsynaptic internalization of GABAARs via dynamin signaling. Therefore, persistent activation of OT neurons following pup calls in vivo is likely mediated by disinhibition. Using a genetically-encoded OT sensor (Mignocchi et al., 2020), we demonstrated that pup calls, but not pure tones, were efficient in triggering OT release in downstream areas such as the VTA. Finally, inhibiting PIL projections to hypothalamus with chemogenetics, perturbed pup retrieval behavior in dams by decreasing the amount of pups retrieved and increasing the latency of retrieval over multiple trials. These findings suggest that the thalamus-hypothalamus noncanonical auditory pathway may be a specific circuit for the detection of social sounds, important for disinhibiting OT neurons, gating OT release in downstream brain areas, and sustaining maternal performance over time.

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Poster

P596. Neuroendocrine Anatomy and Physiology

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Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P596.06

Topic: F.03. Neuroendocrine Processes

Support: NIH R01 MH114994

Title: Oxytocin-paired anogenital stimulation in neonatal mice

Authors: N. M. FERNANDEZ, E. R. FORTI, *E. HAMMOCK;
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Abstract: Oxytocin (OXT) is a neuropeptide best known for its roles in parturition, lactation, and maternal care, and the modulation of adult social behavior. OXT is present in maternal saliva and OXT receptors (OXTR) are present in the external anogenital area of male and female neonatal mice. Studies in rats have demonstrated the importance of anogenital licking and grooming of pups by dams during development, assisting in urination and defecation and

influencing typical neurodevelopmental outcomes. Artificial anogenital stimulation of rat and rabbit neonates with a paint brush, mimicking maternal licking and grooming, has led to increased c-Fos immunoreactivity in the hypothalamus. Therefore, the goal of this experiment is to determine if exogenous OXT applied at the peripheral site of anogenital sensory stimulation modulates brain activation in infancy. Male and female post-natal day 8 OXTR-EGFP reporter mice were randomized to treatment group which included a "holding" control or anogenital stimulation delivered by a paint brush dipped in one of three OXT manipulation conditions (saline vehicle, 1 μM OXT, or 1 μM OXTR antagonist — atosiban). Ninety minutes after the stimulation, neonates were perfused with 4% paraformaldehyde and brains processed for c-Fos immunoreactivity. Preliminary analyses indicate that, like in rats and rabbits, anogenital stimulation enhances hypothalamic c-Fos immunoreactivity in neonatal mice. Data collection is ongoing. The final analyses will be sufficiently powered to detect medium to large effect sizes, including treatment × sex interactions.

Disclosures: N.M. Fernandez: None. **E.R. Forti:** None. **E. Hammock:** None.

Poster

P596. Neuroendocrine Anatomy and Physiology

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Program #/Poster #: P596.07

Topic: F.03. Neuroendocrine Processes

Support: NIH Grant R15HD090606

Title: Oxytocin and vasopressin 1a receptors are functional in both sexes at embryonic day 16.5 in mice

Authors: *E. J. SOMMER, H. K. CALDWELL;
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Abstract: There is mounting evidence that the oxytocin (Oxt) and vasopressin (Avp) systems contribute to early brain formation. Specifically, work in transgenic mice as well as the embryonic manipulation of the Oxt system suggests that signaling through the Oxt receptor (Oxtr) during embryonic development affects behavior in adulthood. Unfortunately, at this time, very little is known or understood about what the roles of these systems during embryonic development. What is known is that, in mice, the Oxtr is present in both sexes as early as embryonic day (E) 16.5, while the production of Oxt is temporally offset between females and males. In females, Oxt mRNA is found as early as E12.5, but in males it is not transcribed until postnatal day 2. In contrast, mRNA transcripts for Avp are found as early as E13.5 and the Avp 1a receptor (Avpr1a) is available in both sexes at E14.5. While it is not clear why there would be a receptor without an obvious ligand for the Oxtr in males, the question of what is activating the

Oxtr or the Avpr1a is premature without knowing whether the receptors are even functional during embryonic development. So, in order to assess the functionality of the Oxtr and Avpr1a in both sexes at E16.5, we performed an Oxtr- and Avpr1a-stimulated g-protein coupled receptor binding assay. We hypothesized that the Oxtr and Avpr1a were functional in both sexes and would be stimulated by Oxt and Avp, respectfully. In this assay, functionality was measured by applying an Oxtr or Avpr1a agonist to tissue sections then determining if there is a decrease in the binding of an ^{35}S radiolabeled GTP; which would occur if there was a conformational change in the receptor, resulting in GDP being released from the Gα subunit. We found both that the Oxtr and Avpr1a are functional at E16.5 in both sexes within the same, but specific, brain regions. For the Oxtr, functional binding was observed in the ventricular and subventricular zones of the cortical neuroepithelium, but not the ventricular or subventricular zones of the septal neuroepithelium or the amygdalar area. For the Avpr1a, functional binding was observed in the nucleus of the vertical limb of the diagonal band of Broca, but not the fornix, olfactory tubercle, indusium griseum, or the ventral hypothalamic area. It is important to note that while this experiment cannot provide information about the nature of the ligand utilized in the developing brain, such as Avp acting at the Oxtr or Oxt acting at the Avpr1a, the data do suggest that Oxtr and Avpr1a signaling have the ability to impact embryonic brain development.

Disclosures: E.J. Sommer: None. **H.K. Caldwell:** None.

Poster

P596. Neuroendocrine Anatomy and Physiology

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Program #/Poster #: P596.08

Topic: F.03. Neuroendocrine Processes

Support: USC School of Pharmacy

Title: Cyp11a1 isoforms in the mammalian central nervous system

Authors: *Y. LIN, V. PAPADOPOULOS;
Dept. of Pharmacol. & Pharmaceut. Sci., Sch. of Pharmacy, Univ. of Southern California, Los Angeles, CA

Abstract: Neurosteroids are steroid hormones synthesized by the nervous system, which have essential roles in brain function, neuroinflammation, and neuroprotection. Although the steroid biosynthesis pathway has been assumed to be identical between peripheral steroidogenic organs and the CNS, there are discrepancies between steroidogenic enzyme levels and neurosteroid concentrations in the brain. In particular, cytochrome P450 11A1 (CYP11A1) - the enzyme catalyzing the first step of steroid formation - has been challenging to detect in the human brain even though its product, pregnenolone, is found in high levels in the brain. Although there is one

CYP11A1 found in rats, two CYP11A1 isoforms are present in humans and mice. However, the differences in expression, function, and localization between the isoforms are unknown. Current literature has focused exclusively on CYP11A1 isoform a (i.e., the classical isoform), while isoform b has not been studied.
Given that the classical CYP11A1a isoform has been challenging to detect in the CNS, we hypothesized that the major CYP11A1 isoform found in the CNS might differ from that present in the periphery. qRT-PCR analysis of mouse tissues revealed that the RNA expression of isoform b is approximately two-fold higher in the brain and spinal cord compared to that of isoform a. In contrast, in peripheral steroidogenic tissues in mice, RNA expression levels of *Cyp11a1* isoform a are 100-fold higher than that of isoform b. In the human brain and spinal cord, the RNA expression levels for *CYP11A1* isoform a appear to be higher than those of isoform b. However, there appears to be a truncation in the brain mRNA transcript for *CYP11A1* isoform a localized between exons 1 and 2. No such truncation was seen in the adrenal or testes. To further characterize the localization and function of different isoforms, we transfected CYP11A1 isoforms a and b into MGM-1 human glioma cells. Although both isoforms appear to be localized to the mitochondria, only the classical isoform a is involved in steroidogenesis. Our results suggest that the human brain expresses a CYP11A1 isoform different from the currently known isoforms. The expression of this alternate CYP11A1 isoform may be under different regulatory mechanisms and it may be involved in different functions than the classical CYP11A1 isoform.

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Poster

P596. Neuroendocrine Anatomy and Physiology

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Program #/Poster #: P596.09

Topic: F.03. Neuroendocrine Processes

Support: NIH R01 (DA046064)

Title: Shared genetic susceptibility across multiple types of chronic pain implicates brain and neuroendocrine systems

Authors: *K. ZORINA-LICHTENWALTER[1], C. BANGO[2], M. C. KELLER[1], L. VAN OUDENHOVE[3], M. CEKO[4], M. A. LINDQUIST[5], N. P. FRIEDMAN[1], T. D. WAGER[2]; [1]Univ. of Colorado Boulder, Boulder, CO; [2]Dartmouth Col., Hanover, NH; [3]Dept. of Chronic Dis. and Metabolism and Leuven Brain Inst., KU Leuven, Leuven, Belgium; [4]ICS, Boulder, CO; [5]Dept. of Biostatistics, Johns Hopkins Univ., Baltimore, MD

Abstract: Current conceptualizations of chronic pain rely heavily on body site and symptom-based groupings. However, pain conditions show substantial phenotypic and genetic overlap, which suggests shared underlying pathophysiology. We characterized the genetic component of this shared risk across 24 chronic pain conditions in the UK Biobank dataset (N=500,000). We conducted a genome-wide association study (GWAS) on each condition and estimated genetic correlations (indicative of shared genetic risk) among them. We used confirmatory factor analysis (CFA) within a genomic structural equation modeling (gSEM) framework to compare groupings in three ways: (1) by body site, (2) by putative etiology, and (3) using results derived from exploratory factor analysis (EFA, a data-driven method). The EFA-CFA pipeline was validated using a split-genome approach with model discovery and validation in odd and even autosomes, respectively. As a complement to factor analysis, we subjected the genetic correlation matrix to graph theoretic analyses, characterizing the network properties and community structure. All analyses pointed to a common genetic factor that confers shared risk across pain conditions. GSEM results showed evidence of a bifactor structure, with a prominent general factor explaining most of the shared genetic variance and two body site-specific factors related to musculoskeletal and cranio-visceral disorders. Network visualization revealed a single dominant community of 17 highly interconnected conditions that share genetic associations. Common factor loadings and network betweenness centrality showed arthritis, back, and neck pain to be the top conditions in terms of shared genetic variance. A GWAS on the common factor showed 163 genome-wide significant loci, which we investigated using FUnctional MApping (FUMA) pathway and annotation analysis. FUMA revealed associations with high expression levels in the pituitary gland and several brain tissues -- particularly hippocampus, cerebellum, and nucleus accumbens -- as well as associated gene overlap with behavioral phenotypes: mood, sleep, and cognition. Overall, these results indicate broad genetic vulnerability across pain disorders and suggest involvement of common neural circuits and neuroendocrine pathways.

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Poster

P596. Neuroendocrine Anatomy and Physiology

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Topic: F.03. Neuroendocrine Processes

Support: NIH RO1-NS050465
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NIH R01-NS1168383

Title: Thyroid hormone T4 mitigates TBI-induced accumulation of lipid droplets in liver

Authors: *M. KHANDELWAL[1], G. KRISHNA[1], Z. YING[1], F. GOMEZ-PINILLA[1,2];
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Abstract: Traumatic brain injury is the most common cause of death and disability. Most of existing efforts have been devoted to understand the TBI pathology in the brain without consideration that the periphery can modulate TBI pathogenesis. In particular, given the role of liver in detoxification and processing of lipids and proteins important for brain function, it is crucial to determine the action of liver on TBI pathogenesis. We are following up previous results that TBI influences fatty acid metabolism and that these effects are exacerbated by fructose ingestion. Oil Red O staining revealed a widespread accumulation of large lipids droplets in rats either receiving fructose or TBI injury, and an even larger accumulation in fructose rats exposed to TBI. Lipid droplets in hepatic tissue are involved in the storage of lipids such as triglycerides, steryl esters and retinyl esters. Lipid droplets are crucial for lipid and energy metabolism; however, excessive accumulation of lipid droplets in organelles is linked to various metabolic disorders such as obesity, fatty liver and atherosclerosis. We have studied the action of T4 thyroid hormone based on results of single cell genomics (Gomez-Pinilla and Yang; Nature Communications, 2018) pointing out thyroid hormone as an important metabolic modulator of TBI and a potential therapeutic target. T4 administration significantly reduced the size, intensity and number of lipid droplets. Additionally, T4 administration reduced the LXR II levels (marker for lipid droplets) in fructose fed TBI rats. In fructose fed-TBI animals the levels of Fatty acid synthase (FAS) were increased and this could be the cause of triglycerides accumulation in liver tissue. FAS is a key lipogenic enzyme involved in fatty acid metabolism such that elevated levels of FAS results in accumulation of triglycerides thereby causing oil droplet formation. We observed significant reduction in FAS levels in T4 administered animals. Thus, thyroid hormone (T4) could be a promising therapeutic agent in treating individuals suffering from TBI and consume high fructose diet.

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Poster

P596. Neuroendocrine Anatomy and Physiology

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P596.11

Topic: F.03. Neuroendocrine Processes

Support: Undergraduate Research Creative Project (UROP) award from Wayne State University

Title: A systematic review and meta-analysis on the effects of exercise on the endocannabinoid system

Authors: *S. DESAI[1], B. BORG[1], C. CUTTLER[5], K. M. CROMBIE[6,7], C. A. RABINAK[1,2,3,4], M. N. HILL[8], H. A. MARUSAK[1,4];
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Abstract: The endocannabinoid (eCB) system plays a key role in maintaining homeostasis, including regulating metabolism and stress responses. Chronic stress may blunt eCB signaling, and disruptions in eCB signaling have been linked to stress-related psychiatric disorders and physical health conditions, including anxiety, depression, posttraumatic stress disorder (PTSD), diabetes, and obesity. Pharmacological and non-pharmacological behavioral interventions (e.g., exercise) that target the eCB system may be promising therapeutic approaches for preventing and treating stress-related diseases. Here, we perform a systematic review and the first meta-analysis to examine the impact of exercise on circulating eCB concentrations. Thirty-three articles (reporting on 51 samples) were included in the systematic review, and ten were included in the meta-analysis. The majority of samples that measured anandamide (AEA) (73.5%) showed a significant increase in AEA concentrations following acute exercise, whereas effects on 2-arachidonoylglycerol (2-AG) were inconsistent in the systematic review. However, the meta-analysis revealed a consistent increase in both AEA and 2-AG following acute exercise across modalities (e.g., running, cycling), species (e.g., humans, mice), and in those with and without pre-existing health conditions (e.g., PTSD, depression). There was substantial heterogeneity in the magnitude of the effect across studies, which may relate to exercise intensity, physical fitness, timing of measurement, and/or fasted state. Effects of chronic exercise were inconsistent. Potential interpretations of exercise-induced mobilization of eCBs, like replenishment of energy stores, along with possible implications, like mediating analgesic and mood-elevating effects of exercise, are included. Our review highlights the ability of exercise to activate the eCB system and mobilize eCBs. We offer recommendations for future work and discuss exercise as a behavioral intervention to prevent and treat stress-related psychopathology.

Disclosures: S. Desai: None. **B. Borg:** None. **C. Cuttler:** None. **K.M. Crombie:** None. **C.A. Rabinak:** None. **M.N. Hill:** None. **H.A. Marusak:** None.

Poster

P596. Neuroendocrine Anatomy and Physiology

Location: Virtual Only

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Program #/Poster #: P596.12

Topic: F.03. Neuroendocrine Processes

Support: Univ. of Illinois Graduate College Dissertation Research Travel Grant
Private donations

Title: Novel sexually-differentiated cell populations in the preoptic area of a sex-changing fish, Amphiprion ocellaris, characterized by single-nucleus RNA sequencing

Authors: *C. G. PARKER[1], Z. V. JOHNSON[2], B. E. HEGARTY[2], G. W. GRUENHAGEN[2], J. T. STREELMAN[2], J. S. RHODES[1];
[1]Univ. of Illinois At Urbana-Champaign, Urbana, IL; [2]Georgia Inst. of Technol., Atlanta, GA

Abstract: Across vertebrate species, sex differences in cell number and phenotype in the preoptic area of the hypothalamus (POA) are essential to regulating sex-specific reproductive physiology and behavior. In mammals, male POA differentiation can be traced back to the presence of gonad-derived androgens, while female POA differentiation (feminization) does not have a clear trigger to mark its beginning and proceeds without gonadal influence. As such, POA feminization is less amenable to laboratory manipulation in mammal models, and is generally not as well understood. In anemonefish like the common clownfish *Amphiprion ocellaris*, POA feminization follows a clear trigger and proceeds without gonadal influence, providing a complementary model for the study of POA feminization. Anemonefish display natural male-to-female sex change, and sex change is easily induced in the lab. Previous work in *A. ocellaris* identified a sexually-differentiated cell population in the anterior POA that is more numerous in females than males. In order to identify potential phenotypes for this cell population, and identify other sexually-differentiated characteristics in the anemonefish POA, we carried out single nucleus RNA sequencing on POA-containing brain tissue from mature male (n=6) and female (n=6) *A. ocellaris*. Libraries were prepared from pooled samples and individual fish were recovered (demultiplexed) using scSplit. Clustering produced 49 clusters, which were then classified as representing major cell classes (e.g. excitatory neurons, inhibitory neurons, radial glia, microglia) using canonical markers. After recovering individuals from pooled samples we tested which clusters differed significantly between the sexes on the basis of cell number. We found 12 clusters to be sexually differentiated, of which 7 were female-biased and 5 were male-biased. One of the male-biased clusters was found to be strikingly differentiated (prominent in males and almost absent in females) and contained a high proportion of cells expressing an androgen receptor gene, suggesting a potential role in brain-pituitary-gonad signaling. Possible homology of the identified sexually dimorphic cell populations in the fish POA with mammalian POA will be evaluated by statistical comparisons with mouse POA single cell data (publically available). Results provide clear cellular composition endpoints for sexual differentiation of the POA that is accomplished during sex change. Further studies will explore the time-course, gonad-dependence/independence, signaling cascades, and epigenetic programming involved in active feminization of the POA in the novel anemonefish model.

Disclosures: C.G. Parker: None. **Z.V. Johnson:** None. **B.E. Hegarty:** None. **G.W. Gruenhagen:** None. **J.T. Streelman:** None. **J.S. Rhodes:** None.

Poster

P610. Hypothalamus and Extended Amygdala in Stress, Fear, and Anxiety I

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P610.01

Topic: F.04. Stress and the Brain

Support: EU-H2020-MSCA-ITN-812777

Title: Social acute stress activates different areas in the chicken central extended amygdala

Authors: A. PROSS[1,2], A. H. METWALLI[1,2], J. A. MORENO[1], *L. MEDINA[1,2], E. DESFILIS[1,2];
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Abstract: Stress is a major problem in poultry farms and research animal facilities due to its negative impact on animal growth, reproduction, susceptibility to disease and welfare. Neural regulation of the stress response has been extensively studied in mammals, highlighting a critical role of the central extended amygdala (EAce) as a major controller of the hypothalamic-pituitary-adrenocortical axis and the sympatho-adrenomedullary system. There is high conservation on the hypothalamic and brainstem centers that trigger the stress response between species, but very little is known on the regulation of the stress response by the amygdala in non-mammals. Using an evolutionary developmental biology approach, our group identified several areas in the chicken telencephalic subpallium, which include cell populations likely homologous to those of mammalian EAce. Our goal was to study if the putative EAce of domestic chicken (*Gallus gallus domesticus;* White Leghorn) is involved in the stress response. We used a previously described paradigm of acute social isolation in seven-day-old chickens, which leads to increased distress vocalizations and corticosterone levels, as reliable markers of biological stress. Fertilized eggs were obtained from a farm and incubated in the laboratory at standard temperature, lighting and humidity conditions. After hatching, chicks were housed in groups, in pens, with food and water ad libitum. On day 7, pairs of same sex animals were treated as follows: one was immediately sacrificed, while the other was isolated from the group for 30 minutes and then sacrificed. Brains were rapidly dissected, and samples were collected, using 1mm-diameter punches, from three different EAce areas: lateral bed nucleus of the stria terminalis (BSTL), peri-intrapeduncular island field and oval central amygdala nucleus (pINP-Ov), and capsular central amygdala (CeC). These samples were analysed using quantitative retrotranscription PCR to detect differential expression of the immediate early gene cFos, comparing isolated with control animals. Two different housekeeping genes (GAPDH and HPRT1) were used to normalize the value. We found a significantly higher cFos mRNA expression in BSTL and CeC but not in pINP-Ov in the stressed animals compared to controls. The activation in BSTL and CeC supports a role of these areas in acute social stress. These

results suggest a conservation of function between avian and mammalian EAce, and open the venue for future studies of the specific cell populations that become active following acute social stress.

Disclosures: A. Pross: None. **A.H. Metwalli:** None. **J.A. Moreno:** None. **L. Medina:** None. **E. Desfilis:** None.

Poster

P610. Hypothalamus and Extended Amygdala in Stress, Fear, and Anxiety I

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P610.02

Topic: F.04. Stress and the Brain

Support: NIH Grant DA033386
NIH Grant DA042362
NSF GRFP

Title: Examining sex differences and the role of corticotrophin-releasing factor in how acute stress impacts reward learning and dopamine release in rats

Authors: *M. P. JOHNSTON[1], B. HAMAUEI[2], C. E. STELLY[2], M. J. LEFNER[3], M. J. WANAT[4];
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Abstract: Exposure to stress can exert long-lasting consequences on behavior. Recent findings demonstrate that acute stress elicits a persistent elevation in Pavlovian conditioned responding in male rats. This stress-enhanced reward learning occurs due to elevated reward-evoked dopamine release in the ventral lateral striatum (VLS). However, the stress-related signaling that mediates the impact of acute stress on reward learning is unknown. One likely candidate is corticotrophin-releasing factor (CRF), which is released in the ventral tegmental area (VTA) during stress whereby modulating dopamine-dependent behaviors. While there are known sex differences in CRF signaling and behavioral responses to stress, it is unclear if there are also differences in how acute stress and CRF signaling impacts reward learning and dopamine release. This project has two primary goals: (1) determine the role of CRF signaling in the VTA during stress-enhanced reward learning and (2) identify whether sex differences exist in how acute stress influences reward learning and dopamine release in the VLS. Our preliminary findings indicate that antagonizing CRF receptor signaling within the VTA blunts stress-enhanced reward learning. Ongoing experiments are utilizing *in vivo* fast-scan cyclic voltammetry recordings to examine how stress and the stages of the estrous cycle influence VLS dopamine release and reward

learning. The results of this project will provide valuable insights on the interaction between stress, reward learning, and dopamine release in male and female rats.

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Poster

P610. Hypothalamus and Extended Amygdala in Stress, Fear, and Anxiety I

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P610.03

Topic: F.04. Stress and the Brain

Title: Elucidating the Differences in Underlying Pathways of Varying Types of Acute Stress

Authors: T. SALTZ[1,2], *A. B. LIPSHUTZ[3,2], K. R. ANDERSON[2,4], A. MANGANARO[2,4], D. DUMITRIU[2,4];
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Abstract: Elucidating the underlying neurocircuitry of acute stress may provide insight into the causes and predispositions of many neuropsychiatric disorders. Understanding the neurocircuitry that underlies acute stress will allow greater understanding of how different types of stressors are processed in the brain via the activation of discrete neural circuits. While the amygdala has been implicated in the pathophysiology of acute stress, many of the neural pathways and brain areas that project to the amygdala at the time of acute stress remain unknown. Previous data show significant differences in motor function of C57 mice after undergoing acute stress (velocity of 8.29 ± 0.29 cm/s, n = 132) as compared to their paired controls (velocity of 9.68 ± 0.45 cm/s, n = 62). We thus hypothesize a connection between acute social and psychomotor stress though we predict that the two are processed differently in the brain via discrete projections to the amygdala. In order to evaluate this hypothesis we will perform Acute Social Defeat Stress (ASDS) and Tail Suspension Test (TST) in two separate subject groups to induce social and psychomotor stress, respectively. Using the genetic mouse line, TRAP2, as well as validated retrograde tracing tools we will observe projections to neurons that were active at the time of each behavior. TRAP2 mice have Cre expression under a cFos promoter which can be activated with an injection of tamoxifen. A virus containing floxed, inactive TVA-G proteins will be injected bilaterally into the amygdala of TRAP2 mice. When Cre is expressed and activated in cFos neurons, TVA-G proteins are expressed. Injections of tamoxifen will be administered in a controlled time window prior to the behavioral paradigm in order to activate Cre which will induce the expression of TVA-G proteins in neurons that are active at the time of the behavior. A glycoprotein-deleted pseudorabies virus will be used to retrogradely trace monosynaptic

projections to neurons that were active during behavior in the amygdala in order to determine the upstream neural populations that project to these neurons. The glycoprotein-deleted pseudorabies can only infect cells that have TVA-G expression. Therefore, projections will only be traced in neurons that were active and expressed cFos at the time of behavior. We hypothesize that distinct patterns of projections to the amygdala will be distinguishable in comparing social stress versus psychomotor stress.

Disclosures: T. Saltz: None. **A.B. Lipshutz:** None. **K.R. Anderson:** None. **A. Manganaro:** None. **D. Dumitriu:** None.

Poster

P610. Hypothalamus and Extended Amygdala in Stress, Fear, and Anxiety I

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P610.04

Topic: F.04. Stress and the Brain

Support: Boettcher Foundation Webb-Waring Award

Title: Live versus robotic predatory threat on foraging by bed nucleus of the stria terminalis GABA neurons

Authors: *A. LY, A. BARKER, E. PREVOST, D. MCGOVERN, D. ROOT;
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Abstract: The motivation to venture outside a safe context in search for food, in spite of potential threats, is essential to survival. GABA neural activity in the bed nucleus of the stria terminalis (BNST), is implicated in stress and fear response. However, the underlying relationship between GABA BNST activity and changes in foraging behavior in response to a threat experience is unclear. Here, we compared changes in foraging behavior in response to a live or robotic predator threat. In addition, we utilized chemogenetic inhibition in order to investigate the role of GABA BNST activity in predatory threat response. Male and female VGaT::Cre mice received viral delivery of Cre-dependent FLEX-GFP or FLEX-hM4Di to the anteromedial BNST (AP+0.3,ML±0.6,DV-4.05). Mice were trained on a foraging task to search for food at discrete and incrementally greater distances in a brightly lit arena. After obtaining food, mice learned to retreat into a dark, enclosed “nest” safe context. The mice were then exposed to either a robotic land predator or a live predator positioned in front of a food source at the farthest distance. In our findings, both live and robotic predatory threat increased latency of successful foraging during the threat exposure itself. In the post-48 hour period after threat exposure, while robotic predator threat increased time spent in the safe context, live predator threat did not change time spent in the safe context but instead increased the number of head entries to the foraging zone from the nest, which was decreased with chemogenetic inhibition of

VGaT neurons in the BNST. The results demonstrate similarities between the effects of live and robotic predatory threat on foraging behavior during the threat itself but differential effects on foraging response in the 48 hours after the exposure, of which BNST GABA neurons have a selective regulatory role.

Disclosures: A. Ly: None. **A. Barker:** None. **E. Prevost:** None. **D. McGovern:** None. **D. Root:** None.

Poster

P610. Hypothalamus and Extended Amygdala in Stress, Fear, and Anxiety I

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P610.05

Topic: F.04. Stress and the Brain

Support: NIH Grant R01MH118237

Title: Effect of social defeat on medial amygdala activity during social behavior

Authors: *A. RITGER[1], N. FERRARA[2], J. ROSENKRANZ[3];
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Abstract: The medial amygdala (MeA) regulates social behaviors and responses to social cues. Social stress can contribute to depression and there is evidence for reduced MeA volume and connectivity in depression. Repeated social defeat stress in rats results in negative long-term behavioral outcomes including social avoidance and anhedonia, and increases MeA immediate early gene expression. A clearer understanding of how and when MeA neuronal activity is engaged during social investigation following social stress would help delineate a role of the MeA in stress-induced changes in social behavior. The goal of this study was to determine the effect of social defeat on MeA activity during a social preference test (SoPT) in rats. We hypothesized that repeated social defeat stress would decrease social preference and increase MeA activity during a novel social encounter compared to control. MeA activity was measured using fiber photometry in adult male Sprague Dawley rats during the SoPT prior to and after repeated social defeat stress exposure. The SoPT consisted of a 3-chamber apparatus with a novel conspecific rat and a novel object enclosed in small cages in opposite chambers. We measured social preference as the ratio of time spent investigating the novel rat cage compared to the novel object cage. During social defeat, rats were exposed to 5 days of social defeat with a retired breeder Long Evans rat using the resident-intruder paradigm, and control rats were placed in a transport cage for the same amount of time. Following social defeat, MeA calcium transients

were then quantified during the SoPT during the time the rat spent exploring either the novel rat cage or the novel object cage. While preliminary results did not demonstrate a difference in social preference between social defeat and control groups, we did find that social defeat increased MeA activity while investigating the novel rat compared to novel object and compared to baseline recordings before stress. There was little change in MeA activity in the control group during novel rat or object investigation, or between recordings. These results suggest that the MeA response to social stimuli is increased relative to non-social stimuli following social defeat. This indicates that MeA responsiveness is sensitive to prolonged social stressors and is altered in response to novel social contexts following social stress. These results may help elucidate the neural mechanisms underlying social withdrawal symptoms in patients that have experienced significant social stress.

Disclosures: A. Ritger: None. **N. Ferrara:** None. **J. Rosenkranz:** None.

Poster

P610. Hypothalamus and Extended Amygdala in Stress, Fear, and Anxiety I

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P610.06

Topic: F.04. Stress and the Brain

Support: American Diabetes Association/Pathway to Stop Diabetes 1-17-ACE-31
American Heart Association 18PRE33960254

Title: Hypothalamus-projecting amygdala neurons link glycemic and behavioral responses to stress via the liver

Authors: *K. DEVARAKONDA[1], A. SHTEKLER[1], V. E. LEHMANN[2], M. JIMENEZ-GONZALEZ[1], A. ALVARSSON[3], R. LI[3], R. HAMPTON[3], P. J. KENNY[4], S. STANLEY[3]; [1]Icahn Sch. of Med. at Mount Sinai, New York, NY; [2]Neurosci., [3]Diabetes, Obesity and Metabolism Inst., [4]Dept. of Pharmacol. and Systems Therapeut., Icahn Sch. of Med. At Mount Sinai, New York, NY

Abstract: When faced with a threat, animals must rapidly mobilize energy stores to support adaptive behavioral responses. Many brain regions mediate the autonomic, endocrine, and behavioral responses to stress. Limbic system brain sites such as the prefrontal (cingulate) cortex, amygdala, and hippocampus coordinate behavioral responses to acute and chronic stressors in a top-down manner by transmitting stress-relevant sensory, emotional, social, and environmental information to hypothalamic and hindbrain stress regions. In contrast to hypothalamic and hindbrain circuits, little is currently known about limbic contributions to glycemic and metabolic responses to stress or their involvement in maintenance of blood glucose homeostasis during non-stress periods. This is important because limbic system dysfunction is

heavily implicated in stress-induced mood disorders, which in turn are associated with increased risk of type II diabetes (T2D) and other abnormalities in blood glucose homeostasis. Here, we investigated the role of the medial amygdala (MeA) in coordinating metabolic responses to stress. First, we show that restraint and fasting stress recruit neural activity in anterior and posterior subdivisions of the MeA. Then, using anterograde and retrograde viral tracers, we identify a population of stress-responsive MeA neurons that project to the ventromedial hypothalamus (VMH). Chemogenetically stimulating VMH-projecting MeA neurons induces stress-like hyperglycemia and facilitates counterregulatory responses to fasting stress whereas their selective lesioning attenuates the hyperglycemic response to psychosocial stressors and impairs the counterregulatory response to an insulin challenge. Importantly, we show that these stress-related glycemic actions of VMH-projecting MeA neurons occur independent of changes in adrenal and pancreatic glucoregulatory hormones and instead operate through their polysynaptic connections to the liver via the celiac ganglia. Together, our findings identify a amygdala-regulated limbic-hepatic circuit that coordinates glycemic responses to stress.

Disclosures: K. Devarakonda: None. **A. Shtekler:** None. **V.E. Lehmann:** None. **M. Jimenez-Gonzalez:** None. **A. Alvarsson:** None. **R. Li:** None. **R. Hampton:** None. **P.J. Kenny:** None. **S. Stanley:** None.

Poster

P610. Hypothalamus and Extended Amygdala in Stress, Fear, and Anxiety I

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P610.07

Topic: F.04. Stress and the Brain

Support: BrainsCAN Accelerator Grant #R5316A15

Title: Divergent electrophysiological and morphological properties of hypothalamic paraventricular nucleus neurons between the common marmoset and the mouse

Authors: *J. K. SUNSTRUM[1,3], S. A. MESTERN[1,3], R. PRZY[1], S. EVERLING[2,3,4], J. C. MARTINEZ-TRUJILLO[2,3,4], W. INOUE[2,3,4];
[1]Neurosci., [2]Physiol. and Pharmacol., Western Univ., London, ON, Canada; [3]Robarts Res. Inst., London, ON, Canada; [4]Brain and Mind Inst., London, ON, Canada

Abstract: The release of stress hormones via the hypothalamic-pituitary-adrenal (HPA) axis is thought to be preserved across mammals. The apex of the HPA axis is formed by parvocellular neuroendocrine neurons in the paraventricular nucleus of the hypothalamus (PVN) that release corticotropin releasing hormone (CRH). While PVN-CRH neurons have been extensively studied in rodents, studies in primates remain scarce. It is possible that the diurnal activity cycle and complex social lives of primates have exposed them to different stressors, and PVN-CRH

neurons have evolved new features under evolutionary pressures. In this exploratory study, we compared PVN neurons between mice and the common marmoset (Callithrix jacchus), a New World primate that has emerged as a promising model in neuroscience. Using patch clamp electrophysiology in acute brain slices, combined with post-hoc morphology reconstruction and immunohistochemistry, we characterized marmoset PVN neurons (N = 10, n= 252) and compared them to their mouse counterparts. Marmoset PVN parvocellular neurons showed stereotyped electrophysiological features well-established in rodents and were distinct from other PVN neuron-types. However, marmoset PVN parvocellular neurons exhibited substantially larger sag-current and shifted membrane resonance due to hyperpolarization-activated cyclic nucleotide-gated (HCN) channels. Interestingly, female (N = 5) parvocellular neurons exhibited greater voltage sag compared to male in marmosets but not mice. Further, morphological analysis revealed that marmoset PVN parvocellular neurons exhibit more dendritic spines compared to mice. These distinct features of marmoset PVN parvocellular neurons may reflect fundamental differences in synaptic integration adaptive for the lifestyle and stressors of primates.

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Poster

P610. Hypothalamus and Extended Amygdala in Stress, Fear, and Anxiety I

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P610.08

Topic: F.04. Stress and the Brain

Support: MH97988 (SEH)

Title: Pituitary adenylate cyclase activating polypeptide (PACAP) neurons in the bed nucleus of the striaterminalis (BNST) and their projections

Authors: *M. AKTAR[1], L. LEPEAK[2], V. MAY[4], S. E. HAMMACK[3];
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Abstract:
Pituitary adenylate cyclase activating polypeptide (PACAP) neurons in the bed nucleus of the stria terminalis (BNST) and their projections Mahafuza Aktar, Lauren Lepeak, Victor May and Sayamwong E. Hammack
Abstract
Pituitary adenylate cyclase activating polypeptide (PACAP) is a highly conserved neuropeptide playing pivotal roles in numerous physiological functions. We have demonstrated that PACAP

release in the bed nucleus of the stria terminalis (BNST) is critical for many of the behavioral and physiological consequences of stressor exposure. BNST PACAP may be signalingfrom several sources, including afferent fibers that originate in the lateral parabrachial nucleus (LPBn) as well as local PACAP-positive neurons residing within subregions of the BNST. Our prior in-situ hybridization studies have suggested that local PACAP production may be increased following chronic stress, and the activation of BNST PACAP neurons may play roles in coordinating behavioral and physiological stress responses. To determine the projection targets of these local BNST PACAP neurons, we infused AAV/EF1a-DIO-mCherry reporter vector into the BNST region of PACAP-ires-Cre mice to identify BNST PACAP-expressing neurons. Consistent with our previous work, we identified robust PACAP expression in local BNST neurons. PACAP neurons were found in anterior, posterior and ventral regions of the BNST and we traced some of their long distance fibers projections through the stria terminalis to amygdala subregions. Apart from the amygdala, we also identified novel BNST PACAP projections to the anterior forebrain and the medial habenula (MHb). These observations support the literature suggesting BNST PACAP activation is critical for many of the consequences of stressor exposure, and further clarify the neural circuits involved.

Disclosures: M. Aktar: None. **L. Lepeak:** None. **V. May:** None. **S.E. Hammack:** None.

Poster

P671. Alcohol Intake and Preference

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P671.01

Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA U01AA025932
NIAAA R01AA021505

Title: Chronic alcohol intake compromises cholinergic modulation of striatal glutamatergic transmission and reduces behavioral flexibility

Authors: *Z. HUANG[1], T. MA[1], X. XIE[1], Y. CHENG[1], X. ZHUANG[1], H. GANGAL[1], X. WANG[1], L. SMITH[1], R. SMITH[2], Y. ZHOU[3], J. WANG[1];
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Abstract: Exposure to addictive substances impairs flexible decision-making. Striatal cholinergic interneurons (CINs) are associated with behavioral flexibility. However, whether and how chronic alcohol intake alters behavioral flexibility and CIN-mediated control of striatal output remain unclear. Here, we report that chronic alcohol consumption and withdrawal impairs

reversal of instrumental learning. The alcohol-mediated behavioral inflexibility is associated with several electrophysiological alterations. First, the spontaneous firing of CINs was increased by chronic alcohol consumption. Second, chronic alcohol intake reduced excitatory inputs from the thalamic parafascicular nucleus to CINs in the dorsomedial striatum (DMS). Thalamic excitation triggers a burst-pause response in DMS CINs, which is critical for reversal learning. Third, the burst-pause responses of CINs were also altered by chronic alcohol intake. Moreover, chronic alcohol consumption impaired CIN-mediated inhibition of glutamatergic transmission in dopamine D1 receptor-expressing medium spiny neurons (D1-MSNs), which drive "Go" actions, and impaired CIN-mediated facilitation of glutamatergic transmission in D2-MSNs, which control "No-Go" behaviors. These results demonstrate that chronic alcohol intake reduces thalamic inputs to striatal CINs, compromising their regulation of glutamatergic transmission in MSNs, which may contribute to alcohol-mediated behavioral inflexibility. These results provide a potential neural mechanism underlying alcohol use disorder.

Disclosures: Z. Huang: None. **T. Ma:** None. **X. Xie:** None. **Y. Cheng:** None. **X. Zhuang:** None. **H. Gangal:** None. **X. Wang:** None. **L. Smith:** None. **R. Smith:** None. **Y. Zhou:** None. **J. Wang:** None.

Poster

P671. Alcohol Intake and Preference

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Program #/Poster #: P671.02

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant AA027915
NIH Grant NS118727
Miami University Office of Research for Undergraduates

Title: Investigation of cortical and striatal dopamine contributions to binge- and compulsive-like alcohol intake

Authors: *E. A. SNEDDON, K. M. SCHUH, A. M. NADER, M. A. MUENCH, B. M. MASTERS, K. A. FENNELL, N. SHAND, S. C. MONROE, A. K. RADKE;
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Abstract: Binge- and compulsive-like alcohol intake are markers of alcohol use disorder. Dopaminergic projections and mesocorticolimbic structures such as the medial and lateral orbitofrontal cortices (mOFC; lOFC), the ventral tegmental area (VTA), and nucleus accumbens core (NAcc) have all been implicated in alcohol drinking behaviors. Here we used chemogenetics and knockout mice to determine the roles of the mOFC, lOFC, VTA-NAcc projection, and dopaminergic contributions to ethanol (EtOH) drinking behaviors. Experiment 1:

The viral vector, AAV-hSn-hM4D(Gi)-mcherry was injected into the mOFC (n = 20) or lOFC (n = 15). Infected neurons expressed the Designer Receptor Exclusively Activated by Designer Drugs hM4Di. Clozapine-N-oxide (CNO) binds to hM4Di to cause neural inhibition. Post-surgery, mice self-administered 15% EtOH and water in a limited access drinking in the dark paradigm for 2 h/day. Escalation took place across 12 sessions. CNO was given to mice on session 11 to assess the effects of neuronal inhibition on EtOH drinking. Quinine (100 µM) was added to the EtOH bottle on sessions 13 - 15. Mice randomly received either CNO or vehicle (VEH) injections on sessions 13 or 15 in a crossover design. Experiment 2: The retrograde viral vector, AAV-hSn-DIO-hM4Di (Gi) was injected into the NAcc of DAT-Cre transgenic mice (n = 28, Cre+ = 16, Cre- = 12) to allow neuronal inhibition in dopaminergic neurons projecting from the VTA to NAcc. Mice were tested in the same paradigm described in Exp. 1. Experiment 3: A DAT knock-out (KO) transgenic line of mice (n = 29, HET = 17, WT = 12) self-administered 15% EtOH or water in a 48-h continuous access paradigm across 10 drinking sessions. Mice self-administered 15% EtOH for 24 h for one baseline day, then, quinine was added to the EtOH bottle in escalating concentrations: 12.5, 25, 100, and 250 µM. Each concentration of quinine was presented for four 24-h sessions, then mice were re-exposed to 15% EtOH for three 24-h sessions between each quinine presentation. For Exp. 1, inhibition of the mOFC did not alter binge- or compulsive-like EtOH intake. Inhibition of the lOFC increased consumption of 15% EtOH but not quinine + EtOH. For Exp. 2, preliminary data suggest that inhibition of the VTA-NAcc does not alter EtOH intake but may increase consumption of quinine + EtOH ($p = 0.067$). For Exp. 3, no differences between groups were observed on EtOH- or compulsive-like intake. All mice showed compulsive-like intake at 12.5 and 25 µM quinine. These results validate the role of the lOFC in the regulation of EtOH drinking and suggest a role for dopaminergic projections from the VTA to NAcc in compulsive-like EtOH drinking.

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Poster

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Program #/Poster #: P671.03

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA042737

Title: Adolescent-onset ethanol drinking produces long-lasting COX-2-mediated changes on methamphetamine relapse in female adolescent rats.

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Abstract: Ethanol (EtOH) and Methamphetamine (Meth) are co-abused drugs but little is known about how they interact to affect behavior. Existing models of co-abuse employ EtOH self-administration and investigator-administered Meth in adult males, but few studies have examined adolescent-onset EtOH drinking and chronic Meth self-administration in female populations. This study aimed to determine the effects of EtOH drinking on later Meth abuse and the role of cyclo-oxygenase 2 (COX2), the enzyme involved in the synthesis of prostaglandins, in serial drug use in female adolescent rats. Rats drank EtOH or water (H2O) for 21 days during adolescence (PD28-49) followed by 21 days of Meth or saline self-administration via jugular vein catheter (PD49-70). Rats then underwent a 7-day Meth abstinence before a cue-primed Meth seeking challenge. Rats were then euthanized and brains were analyzed for changes in COX-2 by Western blot. Prior EtOH drinking had no effect on Meth self-administration. In the cue-primed Meth seeking test, H2O/Meth rats (n=10) pressed a lever previously associated with Meth 124±11.2 times (Mean±SEM) during challenge day, while EtOH/Meth rats (n=10) pressed 91.3±11 times, a 26.3% reduction ($p<0.05$; 2-way ANOVA). EtOH/Meth rats (n=10) had a 73.8% increase in COX-2 immunoreactivity in the striatum compared to H2O/Meth rats (n=9) ($p<0.05$). In a subsequent experiment, adolescent female rats underwent the same serial drug exposure protocol but were treated with the COX-2 inhibitor nimesulide or vehicle during the 7-day Meth abstinence period. In the cue-primed Meth seeking challenge, EtOH/Meth rats that were treated with nimesulide (n=9) had 97.2±9.7 presses, approximately equivalent to H2O/Meth/nimesulide rats (n=9) that pressed 99.5±9.7 times. Both nimesulide treated groups had significantly elevated pressing compared to H2O/Meth/vehicle (n=8, 73.2±9.3 presses) and EtOH/Meth/vehicle (n=8, 80.3±11.1 presses) ($p<0.05$). Overall, EtOH drinking during adolescence attenuated cue-primed drug seeking behavior and produced a long-term increase in COX-2 in the striatum. Conversely, nimesulide inhibition of COX-2 during Meth abstinence restored cue-primed Meth seeking in EtOH drinkers to a rate equivalent with that of H2O drinking rats implicating a role for COX-2 during abstinence from Meth on cue-primed Meth seeking. Based on the role of prostaglandin synthesis in inflammation, future studies will examine how EtOH drinking during adolescence contributes to possible changes in microglia.

Disclosures: H.L. Kline: None. **B.K. Yamamoto:** None.

Poster

P671. Alcohol Intake and Preference

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Program #/Poster #: P671.04

Topic: G.09. Drugs of Abuse and Addiction

Support: PAPIIT-DGAPA 201420
CONACYT 1085633

Title: Effect of long-term permanent sugar consumption on ethanol preference in adolescent and adult rats

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Abstract: The exacerbated sugar consumption and its cognitive processing involve the activation of the brain's reward system that mediate learning and behavior related to reinforcing the consumption of sweet and addictive substances. However, few studies have analyzed the impact of persistent sugar consumption and its relationship with the intake of addictive substances, such as ethanol, mainly when it is present in sugary alcoholic mixtures. On the other hand, it is known that the late maturation of frontal cortical areas during adolescence is related to the early intake of addictive substances such as ethanol. Given that alcohol intake usually begins in adolescence through sugary alcoholic mixtures, the objective of this study was to evaluate, in adolescent and adult rats, the effects of prolonged and permanent sugar exposure on the later preference for sugary ethanol.Male and female adolescent and adult Wistar rats were housed individually and kept at 23°C, in a reverse light-dark cycle. Food was available ad libitum. Rats were randomly assigned to the groups: Naive (N) or Chronic (C), with access to water or sugar, respectively, as the only liquid available for 24 h/21 days. Bodyweight, fluid, and food intake were recorded daily. After 21 days, the chronic group was divided into a group with continuous access to sugar (CS) and a group deprived of sugar, with access only to water (CD), between each of the four sugary ethanol (10%ethanol-10% sugar) vs. water preference tests (24 h each). In groups CS and CD adolescent and adult of both sexes, the chronic sugar consumption induced a suppressive intake effect of the sugary ethanol solution (S-EtOH); however, only the adolescent CD rats increased their consumption of sugar-ethanol mixture along with the preference tests. Furthermore, the data of adult rats also exposed to S-EtOH during adolescence (e.g., double exposure), the deprivation effect lasts into adulthood; since the CD double exposure adult consumed more S-EtOH than the CD adults that only consume S-EtOH in adulthood. Altogether, these results suggest that adolescent rats are more susceptible to sugar deprivation, independently of sex. The susceptibility could be related to late brain maturation and its implications on increased impulsive and risk-seeking behaviors such as the initiation in the use of addictive substances. Moreover, sugary ethanol mixture consumption during adolescence, both by females and males deprived of sugar, can facilitate its intake during adulthood.

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Poster

P671. Alcohol Intake and Preference

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Program #/Poster #: P671.05

Topic: G.09. Drugs of Abuse and Addiction

Support: NIGMS GM113131

Title: Assessment of Grouped and Individual Effects in Coadministration of Nicotine and Ethanol using Behavioral Economics Approach

Authors: *T. W. ALLEN[1], C. L. ROBISON[1], V. M. MADORE[1], N. COVA[1], H. MANNING[1], E. O'KEEFE[1], A. KALINOWSKI[1], J. MULLIGAN[1], K. BABINA[1], B. STARR[1], S. T. BARRETT[2], S. CHARNTIKOV[1];
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Abstract: Alcohol and nicotine are two commonly coadministered and abused legal substances. Estimates place global annual deaths at 3 and 8 million for alcohol and nicotine respectively. Global economic burden of alcohol is estimated at 210-665 billion USD whereas that of nicotine is estimated 422 billion USD. Studies indicate that 80-90% of individuals with alcohol use disorder also smoke cigarettes and tend to smoke more than those without alcohol use disorder. Although recent evidence indicates that nicotine potentiates reward value of appetitive stimuli in animal models, the relationship between alcohol and nicotine use is not fully understood. To address this, we designed a study with two objectives in mind. First, to determine the economic demand for nicotine and ethanol alone and then assess the relationships between two demand variables using grouped and individual levels of assessment. Second, to assess the effect of each substance on consumption of the other substance. Our findings show that overall, rats had highest initial demand for sucrose (*essential value*), followed by sweetened ethanol, ethanol alone, and nicotine. The alpha parameter for nicotine was highest, followed by ethanol, sweetened ethanol, and sucrose. Contingent but not non-contingent access to ethanol decreased nicotine self-administration. Both contingent and non-contingent nicotine administration increased ethanol self-administration. Importantly, parameters derived from individual demand for nicotine predicted responding for ethanol when nicotine was available contingently. This study demonstrates how coadministration of substances can be assessed on grouped and individual levels using behavioral economics approach. Future research needs to further explore how behavioral economics approach can be used to define substance use vulnerable phenotype and response to treatment on individual level.

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Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant AA027915
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Title: Sex differences in punished EtOH responding in an operant paradigm

Authors: *K. A. FENNELL[1], E. A. SNEDDON[2], J. DEMEDIO[3], S. BHATI[3], J. SETTERS[3], S. C. MONROE[3], K. M. SCHUH[4], N. SHAND[3], J. HOFFMAN[3], A. K. RADKE[5];
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Abstract: Alcohol Use Disorder (AUD) is the third leading cause of preventable deaths in the United States, and causes an estimated number of 95,000 deaths in the United States per year. AUD has been shown to have more severe negative consequences for women. Thus, it is necessary to investigate mechanisms that may contribute to sex differences that may arise in alcohol drinking. Compulsive alcohol seeking, a characteristic of AUD, has been modeled in rodents with paradigms that punish drinking with aversive stimuli. Previous studies have found that females are more resistant to quinine punishment when seeking ethanol (EtOH) in an operant paradigm but sex differences in responding for EtOH punished with foot-shock have not been assessed. Thus, we examined whether male and female mice differ in foot-shock-resistant EtOH seeking and the role of mGluR5 receptors in the NAc core in mediating sex differences in punishment resistance. To test for sex differences, C5BL/6J mice were food-restricted to 85% of their free-feeding weight and trained to respond for a 10% sucrose solution on a fixed ratio 1 (FR1) schedule in an operant chamber. Once mice learned to respond, they were transitioned to a fixed ratio 3 (FR3) sucrose fading procedure with 3-5 days of 10% sucrose, 10% sucrose and 10% EtOH, 5% sucrose and 10% EtOH, and 10% EtOH solutions. When responding for the 10% EtOH solution was stable, a foot-shock (0.25 or 0.35 mA) was delivered. A second group followed the same sucrose fading procedure but was administered a 0.35 mA foot shock. A third, group self-administered 10% only and was punished with a 0.25 mA foot-shock. Males and females continued to respond for EtOH but reduced responding for sucrose when a 0.25 mA shock was delivered. Furthermore, males reduced response rates at the 0.35 mA shock while females did not. Additional follow-up experiments suggest these effects are not due to sex differences in foot-shock sensitivity. In a final experiment, mice were implanted with chronically indwelling cannulae in the NAc core and trained to respond for 10% EtOH as described above. The mGluR5 receptor antagonist, MTEP, or vehicle (VEH) was infused prior to delivery of a 0.25 mA foot-shock. Analysis of the effects of MTEP on punishment resistance is ongoing. Together, these results demonstrate that females are more likely to participate in punished EtOH

seeking. Glutamatergic signaling in the NAc core contributes to compulsive-like alcohol drinking and may drive the sex differences observed in this behavior.

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Poster

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Title: Effects of Sex Chromosomes on Compulsive-Like Alcohol Drinking in Four Core Genotype Mice

Authors: *B. M. MASTERS, E. A. SNEDDON, K. A. FENNELL, J. DEMEDIO, L. RASZIER, A. K. RADKE;
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Abstract: Approximately 15 million people in the United States suffer from Alcohol Use Disorder (AUD), 5.5 million of whom are women. Research has shown females to be more susceptible to AUD and to aversion-resistant alcohol consumption compared to males. Yet despite the high prevalence of women with AUD, female vulnerability to alcohol is understudied. Compulsive alcohol seeking is a defining characteristic of AUD that has been modeled using an aversion resistance paradigm known as Drinking in the Dark (DID) in which the bitter tastant quinine is added to ethanol (EtOH). To investigate mechanisms of sex differences in aversion-resistant drinking, we used the Four Core Genotypes (FCG) mouse model. This model includes four genotypes, two of which do not express the Sry gene and develop ovaries (XX/Sry- and XY/Sry-) and two of which express the Sry gene and develop testes (XX/Sry+ and XY/Sry+). In experiment 1, female (Sry-) FCG mice (n = 14, XX/Sry- = 7, XY/Sry- = 7) self-administered 15% EtOH or water in a 2-bottle limited access DID paradigm for four hours each session totaling 15 sessions. Each session began three hours into the dark phase of the mouse circadian cycle. Quinine was then added to the 15% EtOH solution in increasing concentrations of 100 µM, 250 µM, and 500 µM for 5 sessions at each concentration. During each session, bottles were weighed at the 0 h, 30 min, 2 h, and 4 h marks. Estrous cycle monitoring was conducted for 4 sessions at each quinine concentration. As a control, mice self-

administered a 2.5% sucrose solution about 2 weeks after the last EtOH + quinine session. Quinine was then added to the 2.5% sucrose solution in increasing concentrations of 100 µM, 250 µM, and 500 µM for 1 session at each concentration. A water control was conducted using this same DID procedure in a subset of mice who self-administered either plain water or water with quinine. Quinine was added to the second water bottle in increasing concentrations of 100 µM, 250 µM, and 500 µM for 1 session at each concentration. For experiment 2, the same procedure was followed using male (Sry+) FCG mice (n = 14, XX/Sry+ = 7, XY/Sry+ = 7), excluding estrous monitoring. Results of experiment 1 showed that the XY chromosome compliment promotes aversion-resistant EtOH consumption and EtOH preference in mice with ovaries (Sry-). Results of experiment 2 showed that XY/Sry+ mice consume more EtOH overall than XX/Sry+ mice. Although the mechanisms that underly female vulnerability to compulsive drinking are still unknown, these data can be used in future research to investigate potential genetic contributors to sex differences in alcohol drinking behaviors.

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Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIGMS GM113131

Title: Individual response to intermittent-swim stress predicts economic demand for ethanol in male rats

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Abstract: Worldwide, alcohol use disorder results in 3 million deaths every year, which translates to 5.3 % of all deaths. Risk factors like physical or emotional trauma have been previously linked to alcohol consumption. However, not everyone responds the same way to stress; there is evidence of both individual vulnerability as well as individual resilience to stress. With that in mind, our study was designed to test whether the magnitude of individual response to stress can predict a magnitude of economic demand for self-administered ethanol. Wistar rats were trained to self-administer sucrose, sweetened ethanol, and ethanol alone prior to the exposure to stress, and the economic demand was derived for each substance. Rats were then subjected to intermittent-swim stress (n=32) in cold water or sham-stress (n=10) exposure. Ethanol demand was reassessed after a 10-day incubation period. In addition, plasma

corticosterone levels were assessed before and immediately after the traumatic stress exposure. Our main finding shows that a magnitude of corticosterone response to a traumatic stressor predicted an increase in economic demand for ethanol following a traumatic stress episode. We also show that the initial economic demand for ethanol in combination with stress-induced corticosterone response explain 54 % of the variance in economic demand for ethanol after the exposure to stress episode. Our study demonstrates for the first time the utility of the behavioral economics approach in assessing individual response to traumatic stress and stress-induced alcohol consumption. We also demonstrate for the first time that stress can significantly change established ethanol taking with rats vulnerable to stress exhibiting higher levels of post-stress consumption.

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Poster

P939. Electrophysiology Methods: Electrode Arrays I

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Program #/Poster #: P939.01

Topic: I.04. Physiological Methods

Title: Optimized analog filters for power efficient spike detection

Authors: *K. M. BOERGENS[1], A. P. SINGH[1], R. EDGINGTON[1], A. KHAN[1], P. OREL[1], K. NISHIMURA[1], T. STERN[1], R. R. RACZ[2], M. KOLLO[2], P. GAO[3], A. BABAIE-FISHANI[3], S. VEIJALAINEN[3], B. LUYSSAERT[3], A. KEEFE[3], Y. KONG[1], B. DIERICKX[3], A. T. SCHAEFER[2], V. GILJA[4], K. SAHASRABUDDHE[1], M. R. ANGLE[1];
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Abstract: Two predominant paradigms exist for processing electrical recordings of neuronal action potentials. The first aims to preserve raw data; in this paradigm the signal is amplified, filtered, and digitized, after which advanced signal processing is performed in the digital domain. This approach is energetically expensive because it involves digitizing and transmitting high bandwidth data. The second approach is targeted toward motor-prosthetics; in this approach signals are amplified, filtered, and then thresholded to create a binary stream of events. That processing can be performed in the analog domain, which can greatly reduce total power consumption, but it comes at the expense of spike waveform information.Here we describe a third possible approach that preserves spike waveform information but digitizes only a fraction of the raw data. We have implemented an optimized set of analog filters for detecting action potentials from intracortical electrode recordings. The filters are designed using Principal Component Analysis, based on a library of previously recorded spike data, and are instantiated in a CMOS analog circuit that fits within a 125 µm by 250 µm area and consumes 2.0 µW per

channel. The resulting output of n filters represents an n-rank approximation of the signal where each output is a continuously varying PC score, which is sampled at the moment when the spike peaks. This scheme reduces Spike Error Rate as compared to thresholding the output of 2nd order bandpass filters, especially in noisy conditions. We also show that 3 PC score outputs provide enough information to allow for spike sorting that is indistinguishable from that performed by experts on raw data. In conclusion, this technology will enable a new generation of brain-machine interfaces that are less subjected to trade-offs between data quality and power consumption.

Disclosures: K.M. Boergens: A. Employment/Salary (full or part-time):; Paradromics. **A.P. Singh:** A. Employment/Salary (full or part-time):; Paradromics. **R. Edgington:** A. Employment/Salary (full or part-time):; Paradromics. **A. Khan:** A. Employment/Salary (full or part-time):; Paradromics. **P. Orel:** A. Employment/Salary (full or part-time):; Paradromics. **K. Nishimura:** A. Employment/Salary (full or part-time):; Paradromics. **T. Stern:** A. Employment/Salary (full or part-time):; Paradromics. **R.R. Racz:** None. **M. Kollo:** None. **P. Gao:** A. Employment/Salary (full or part-time):; Caeleste. **A. Babaie-Fishani:** A. Employment/Salary (full or part-time):; Caeleste. **S. Veijalainen:** A. Employment/Salary (full or part-time):; Caeleste. **B. Luyssaert:** A. Employment/Salary (full or part-time):; Caeleste. **A. Keefe:** A. Employment/Salary (full or part-time):; Caeleste. **Y. Kong:** A. Employment/Salary (full or part-time):; Paradromics. **B. Dierickx:** A. Employment/Salary (full or part-time):; Caeleste. **A.T. Schaefer:** F. Consulting Fees (e.g., advisory boards); Paradromics. **V. Gilja:** F. Consulting Fees (e.g., advisory boards); Paradromics. **K. Sahasrabuddhe:** A. Employment/Salary (full or part-time):; Paradromics. **M.R. Angle:** A. Employment/Salary (full or part-time):; Paradromics.

Poster

P939. Electrophysiology Methods: Electrode Arrays I

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Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P939.02

Topic: I.04. Physiological Methods

Support: CIHR
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BrainsCAN

Title: A method for chronic electrode implantation in deep brain structures using neuronavigation

Authors: *B. MAHMOUDIAN[1], H. DALAL[6], J. LAU[2], B. W. CORRIGAN[3], K. BARKER[4], A. RANKIN[4], T. PETERS[5], J. C. MARTINEZ-TRUJILLO[7];

[1]Schulich Sch. of Med. and Dent., [2]Sch. of Biomed. Engin., [3]Neurosci., [4]Robarts Res. Inst., [5]Biomed. Engineering, Med. Biophysics, Univ. of Western Ontario, London, ON, Canada; [6]Monash Univ., Melbourne, Australia; [7]Dept. of Physiol. and Pharmacol. and Psychiatry, Brain and Mind Institute, Univ. of Western On, London, ON, Canada

Abstract: Extracellular recordings from behaving animals allow for investigating functions of brain circuits. While ease of access to the cortical surface allows for precise targeting of most cortical structures, accurate targeting of deep brain regions remains a challenge. In addition, whereas chronic cortical recording methods are well established (Roussy et al., 2020), methods for chronic recording of deep brain regions are scarce. As opposed to acute, chronic methods have the advantage of reducing chances of infection (Dotson et al., 2017), requiring less daily preparation, and allowing for longitudinal monitoring of the same population of neurons (McMahon et al., 2014). Here we describe a chronic and semi-chronic electrode implantation method for targeting of Basal Amygdala (BA) in macaque, utilizing the *Brainsight* neuronavigation toolkit and surgical methods borrowed from human SEEG (Stereoelectroencephalography) procedures. Anatomical images (MRI and CT) of two rhesus macaque were used for selection of implantation trajectories targeting BA, as well as designing of a custom cranial cap. Neuronavigation procedures require the co-registration of subject and image space (e.g. MRI), allowing for real-time navigation of anatomical images during the surgical procedure. To perform this co-registration, cranial cap screws were used as fiducial markers: homologous anatomical landmarks on the subject and the images, which when matched, transform the subject space into image space. Co-registration allowed for matching of pre-determined trajectories using image-guided navigation. Performing a 2.4mm craniotomy along the trajectory provided a minimally invasive entrance to the brain. An anchor-bolt (*Ad-Tech Medical*) was used as a small chamber, allowing a custom-made microdrive (monkey Ma) to be mounted, before passing of a polyimide guide tube to above target. Lastly, a 32 (monkey Ma) or 64 channel (monkey Mi) microwire brush array (*Microprobes*) was inserted by hand through the guide tube and lowered into the BA with the microdrive (monkey Ma) or by hand (monkey Mi).
Post implantation CT scan of each subject, co-registered with the T1 MRI, allowed for assessing of the targeting accuracy. Total targeting error of monkey Ma was 2.32mm (Euclidean distance) from the tip of the electrode (2.2 mm medial-lateral, 0.7 mm anterior-posterior, 0.3 mm superior-inferior), while monkey Mi had a total error of 1.44 mm (Euclidean distance; 0.5 mm medial-lateral, 0.9 mm anterior-posterior, 1.0 mm superior-inferior). In summary, our method provides a minimally invasive protocol for implantation of deep brain structures with an average targeting error of 1.88 mm (n=2).

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Poster

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Program #/Poster #: P939.03

Topic: I.04. Physiological Methods

Title: Optimal High Density Micro-ECoG Design

Authors: *A. SINGH[1], T. STERN[1], R. J. EDGINGTON[1], K. SAHASRABUDDHE[1], M. R. ANGLE[1], V. GILJA[2];
[1]Paradromics, Austin, TX; [2]Electrical and Computer Engin., UCSD, La Jolla, CA

Abstract: For individuals with communication-limiting neurological disorders, surface grid electrocorticography (ECoG) based devices have the potential to enable the development of neural prostheses to help restore communication ability. Recent advances in microfabrication processes allow for higher density ECoG with smaller microscale contacts. This reduction in scale can ease the implantation process and provide a more granular spatial measurement of neurophysiological processes. We evaluate the potential of microscale ECoG by recording neural activity from the cortical surface of anesthetized sheep (N=3) with dense arrays of >20,000 18 µm diameter platinum iridium electrodes. Electrode pitch is 50 µm on average and spans an area over 12x12 mm^2 in the auditory cortex of anesthetized sheep (N=3). Electrode spacing (50µm to 1mm), electrode count (N=10 to 2000) and area coverage (1 to 100 mm^2) parameters are evaluated by subsampling and averaging individual electrode responses to estimate optimal parameters with respect to decoding auditory stimuli. Spectral power features are extracted with a preprocessing pipeline and a regularized, cross-validated linear regression model predicts the frequency of 19 presented pure tones from bandpassed high gamma activity. Decoding performance is evaluated by computing the tonal error in octaves between the tone presented and the tone predicted. When considering electrode pitch for a 7.5mm^2 area array, a minimum tonal error of 0.8 octaves is found at a 100 µm pitch (750 electrodes); tonal error is 1.4 octaves at 300 µm pitch and 2 octaves at 1mm pitch. Increasing array area decreases the density of electrodes needed to achieve similar performance, for example 1 octave tonal error can be achieved at 1 mm pitch with 100 mm^2 area coverage, dropping to 30 mm^2 for 300 µm pitch and area coverage less than 10 mm^2 requires 100 µm pitch density. Electrode contact sizes were simulated by averaging the response of multiple physical electrodes over an area of 8 mm^2. Increasing density and decreasing surface size achieved lower tonal error, with optimal performance (<1.32 octaves) for areas less than 0.3 mm^2 at their highest density. These findings suggest that specific neural states may be estimated with similar fidelity by either covering a larger spatial area with larger, sparsely placed electrode contacts or by covering a smaller spatial area with smaller, sparsely placed electrode contacts. These design tradeoffs are a critical consideration in the design of implantable systems for the development of next-generation brain-computer devices to enable functional restoration for individuals with communication disorders.

Disclosures: A. Singh: A. Employment/Salary (full or part-time):; Paradromics. **T. Stern:** A. Employment/Salary (full or part-time):; Paradromics. **R.J. Edgington:** A. Employment/Salary (full or part-time):; Paradromics. **K. Sahasrabuddhe:** A. Employment/Salary (full or part-time):; Paradromics. **M.R. Angle:** A. Employment/Salary (full or part-time):; Paradromics. **V. Gilja:** A.

Employment/Salary (full or part-time):; University of California, San Diego. 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.; Paradromics.

Poster

P939. Electrophysiology Methods: Electrode Arrays I

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P939.04

Topic: I.04. Physiological Methods

Support: Craig H Neilsen Foundation

Title: Chronic intraspinal neural recording & electrical stimulation with braided multi-electrode probes in rats

Authors: *T. KIM[1], A. P. BORISYUK[1], T. BEZDUDNAYA[1], L. ZHOLUDEVA[3], J. WALKER[1], B. SHERMAN[2], M. R. DETLOFF[1], M. A. LANE[1], P. J. REIER[4], S. F. GISZTER[1]; [1]Neurobio. & Anat., Drexel Univ. Col. of Med., Philadelphia, PA; [2]Drexel Univ. Col. of Med., Phiadelphia, PA; [3]Gladstone Inst., San Francisco, CA; [4]Dept Neurosci, Rm L1-100K, Univ. Florida Col. Med. Brain Inst., Gainesville, FL

Abstract: We have designed a novel braided multi-electrode probe (BMEP) comprised of an ultrafine microwire bundle in a tubular braid form for use as a neural interface to record signals or stimulate neural tissue. The BMEP provides not only high mechanical flexibility to follow tissue motions and deformation, but also 6 ~ 24 channels for recording/stimulation in maximum 200μm diameter. The tubular BMEP has a removable tungsten needle to temporarily increase the stiffness of the braid for better tissue penetration and then restore its high flexibility after removal. Based on this mechanism, the probe-type BMEP is designed for easy insertion and release into any region of the central nervous system (CNS). One example is the spinal cord which, due to its length and flexibility, can frequently generate severe tissue motions which cause conventional electrodes to often severely damage tissue. Historically, only few chronic intraspinal recording trials with single microwires have been successful. In early studies our flexible BMEPs were able to chronically record intraspinal neural signals from freely jumping frogs. We are now pursuing three projects requiring chronic intraspinal implants with BMEPs for recordings or electrical stimulation (E-Stim). One is to investigate the therapeutic potential of combining E-Stim and stem cell transplantation after spinal cord injury (SCI) using a chronically implanted BMEP. To explore feasibility, cervical spinal hemi- contusions were followed with spinal neural progenitors being introduced into the lesion cavity a week later. Next, a 6 x 9.6μm BMEP in 2mm length was perpendicularly inserted into the graft region. Daily E-Stim was performed for 4 weeks (5 days / week). For E-Stim and neural recordings, we use a bidirectional

neural interface, Neurochip 2, which is a portable 2ch recording/3ch stimulation device developed by Fetz et al. (2011, IEEE Trans Neural Syst Rehabil Eng **19**(4): 427-435). We confirmed that the implanted BMEPs performed well for chronic recordings and E-Stim. This project is still on-going. The second project is to test an oblique insertion (35~40°) of 6 x 9.6µm long BMEPs (length:6~10mm) below a spinal transection at T10/11 and chronic intraspinal recording with the obliquely inserted BMEPs at L1/2. The oblique insertion seems more mechanically stable for lumbar cord applications than regular vertical insertions. Histological and electrophysiological tests are in progress. The last project tests changes in intraspinal neural activity before and after a SCI in a cervical area. This project has just been initiated and we are testing the quality of chronic neural signals with BMEPs.

Disclosures: T. Kim: None. **A.P. Borisyuk:** None. **T. Bezdudnaya:** None. **L. Zholudeva:** None. **J. Walker:** None. **B. Sherman:** None. **M.R. Detloff:** None. **M.A. Lane:** None. **P.J. Reier:** None. **S.F. Giszter:** None.

Poster

P939. Electrophysiology Methods: Electrode Arrays I

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Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P939.05

Topic: I.04. Physiological Methods

Support: NIH NINDS R44NS065545

Title: High resolution, transcutaneous peripheral nerve interface for quantitative tracking and visualization of nerve activity

Authors: M. MCKINNON[1], A. PREYER[1], R. BLUM[1], C. K. FRANZ[2], *I. CLEMENTS[1]; [1]BioCircuit Technologies, Atlanta, GA; [2]Biologics, Shirley Ryan Abilitylab, Chicago, IL

Abstract: Improved capabilities for non-invasive sensing and manipulation of neuromuscular activity would advance areas of clinical diagnostics, therapeutic stimulation, and neural prosthetics. Typical electrodiagnostic techniques are technically challenging and involve repeated repositioning of large surface electrodes by a highly trained clinician. Here we describe a multichannel transcutaneous recording and stimulation platform for targeted monitoring and manipulation of human nerves and muscles.
This platform was optimized for high-quality electrophysiological recordings in noisy environments (e.g., hospitals). Flexible, adhesive arrays of hydrogel electrodes were customized to target specific peripheral nerves and muscles. Custom software was written to simplify acquisition and data processing of raw signals. Meaningful physiological parameters (e.g., conduction velocity, nerve trajectory) were extracted from processed data by novel algorithms. We were able to track median nerve activity continuously from wrist to elbow with sufficient

resolution to calculate conduction velocity using a custom recording array. Innovative visualization methods were used to display propagating nerve activity intuitively and succinctly. Another electrode array was designed to conform to the elbow for precise spatial recording of ulnar nerve activity. This configuration could enable localization of focal conduction slowing with relevance to cubital tunnel syndrome. The same platform was used to acquire high density surface EMG recordings from skeletal muscle (soleus recruitment in response to tibial nerve stimulation) as part of a reflex operant conditioning therapy. We also recorded diaphragm contraction in response to phrenic nerve stimulation, which could be used to diagnose phrenic neuropathy resulting from severe COVID-19 infection.
A non-invasive, array-based system for peripheral nerve interfacing and quantitative assessment of nerve/muscle function was designed and validated within a variety of clinically-oriented applications. Ultimately this platform has the potential to advance neuromuscular research, neuromodulation, bioelectronic medicine, and neural prosthetics.

Disclosures: M. McKinnon: A. Employment/Salary (full or part-time):; BioCircuit Technologies. **A. Preyer:** A. Employment/Salary (full or part-time):; BioCircuit Technologies. **R. Blum:** A. Employment/Salary (full or part-time):; BioCircuit Technologies. **C.K. Franz:** None. **I. Clements:** A. Employment/Salary (full or part-time):; BioCircuit Technologies.

Poster

P939. Electrophysiology Methods: Electrode Arrays I

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Program #/Poster #: P939.06

Topic: I.04. Physiological Methods

Support: ERC #679253 (ES)
ISF #638/16 (ES)
ISF #1871/17 (ES)
CIHR-IDRC-ISF #2558/18 (ES)

Title: Dual-sided probes for high spatiotemporal resolution recordings of spiking networks

Authors: *F. WU[1], A. LEVI[2], D. LEE[1], H. E. SLOIN[2], L. SPIVAK[2], S. SOMECK[2], E. STARK[2];
[1]Diagnos. Biochips, Glen Burnie, MD; [2]Tel Aviv Univ., tel aviv, Israel

Abstract: Cortical neuronal networks are responsible for various functions: decoding sensory input, encoding motor action, maintaining memories, and many more. Determining which neurons partake in a common function and what network connectivity patterns facilitate functionality is a major goal of neuroscience research. Planar silicon probes enable linking network activity patterns with cognitive function in intact subjects by simultaneously recording

hundreds of neurons within a local network at high spatiotemporal resolution. However, due to the planar structure of present probes, it is still impossible to record the entire neuronal population within a sphere around the probe.
We developed two types of dual-sided hybrid polyimide-silicon probes. In one design ("Dual-sided64"), each probe consists of two dual-sided shanks, each with 16 electrodes spaced 20 μm apart per side. The second design ("Dual-sided128") includes six dual-sided shanks, and every side is equipped with 10-11 electrodes spaced 15 μm apart. The distance between electrodes on opposite sides is 30 μm. A fiber-coupled LED was attached to every dual-sided shank, and then the entire device was mounted on a microdrive and implanted in a freely-moving CaMKII::ChR2 mouse. Recordings were performed in the neocortex and hippocampus of three mice over several months, and compared to recordings carried out under equivalent conditions using single-sided probes.
The dual-sided probes enabled four advances. First, the number of neurons recorded from a given piece of cortex increased, enabling monitoring a larger fraction of local circuit neurons. Second, dual-sided recordings showed higher density of inter-neuronal connections. Third, dual-sided probes allowed 3D triangulation of neuronal somata. Together with connectivity mapping, an imaging-like reconstruction of network structure and connectivity was achieved, without losing the temporal resolution made possible by electrophysiology. Finally, using the dual-sided probes we simultaneously recorded electrophysiological signature of different cellular compartments of the same neuron (soma, dendrites, and axon) on different sides of the same shank.
Dual-sided probe technology is a step towards monitoring all neurons within a small piece of the brain. The dual-sided probes allow reconstructing a neuronal network deep within the brain of freely-moving subjects at high spatial and temporal resolutions. In the future, dual-sided probes could be used to provide a better understanding of local network activity during performance of cognitive functions including learning, memory, and decision making.

Disclosures: F. Wu: A. Employment/Salary (full or part-time):; Diagnostic Biochips Inc.. **A. Levi:** None. **D. Lee:** A. Employment/Salary (full or part-time):; Diagnostic Biochips Inc.. **H.E. Sloin:** None. **L. Spivak:** None. **S. Someck:** None. **E. Stark:** None.

Poster

P939. Electrophysiology Methods: Electrode Arrays I

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Topic: I.04. Physiological Methods

Support: NIH R21EB029740
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ORAU Ralph E. Powe Junior Faculty Enhancement Award

HHMI Hanna Gray Fellowship
Burroughs Wellcome Fund Postdoctoral Enrichment Program Award

Title: Flexible electrode technologies for mapping sensory-driven cellular and subcellular activity from the cortical surface in awake, behaving animals

Authors: *D. L. GONZALES[1], S. R. PLUTA[2], K. JAYANT[1];
[1]Weldon Sch. of Biomed. Engin., [2]Dept. of Biol. Sci., Purdue Univ., West Lafayette, IN

Abstract: Dendrites exhibit diverse modes of regenerative potentials that shape perception, sensory tuning, and reward reinforcement. In mouse cortex, for example, Layer 5 (L5) pyramidal neuron burst-mode firing drives large, all-or-nothing calcium spikes in the apical dendrites that are critical for stimuli detection and object localization. However, the small size of dendrites renders scalable electrical readouts in behaving animals unfeasible with traditional electrodes. Thus, mapping subcellular activity and its relationship to neural computations and animal behavior remains notoriously difficult. Here, we discuss our efforts to develop a flexible and transparent neural interface with arrays of micro- and nano-scale electrodes tailored to map subcellular activity. Specifically, we hypothesized that a soft probe with high-density, thin-film electrodes would conform to the cortical surface and enable a scalable platform for monitoring activity in L5 apical dendrites near the surface of the brain. In mouse barrel cortex, we recorded both local field potentials and single-unit activity from awake animals during sensory stimulation. To determine the origin of these signals at the brain surface, we used simultaneous depth recordings. Silicon probe recordings across cortical layers revealed that field potentials at the cortical surface largely mirror deep-layer activity; however, specific signatures of the LFP remain unique to the upper cortical layers. Furthermore, preliminary experiments in which we performed simultaneous two-photon calcium imaging allowed for monitoring either somatic or dendritic calcium activity while performing electrophysiology at the cortical surface. These initial results suggest a strong dendritic component (possibly apical dendrite calcium spikes) in the LFP at the cortical surface that can be mapped on a scalable platform across barrels in behaving mice. We discuss key control experiments to ascertain the origin of the surface potential measurements and present design considerations of our technology to enable precision sub-cellular activity mapping with minimal invasiveness.
Funding: This work was supported by the following grants. NIH R21EB029740; HFSP RGY0069; HHMI Hanna Gray Fellowship; ORAU Ralph E. Powe Junior Faculty Enhancement Award; Burroughs Wellcome Fund Postdoctoral Enrichment Program Award.

Disclosures: D.L. Gonzales: None. **S.R. Pluta:** None. **K. Jayant:** None.

Poster

P939. Electrophysiology Methods: Electrode Arrays I

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Program #/Poster #: P939.08

Topic: I.04. Physiological Methods

Support: NIH Grant R01NS091236
NIH Grant R41MH113252
NIH Grant R43DA051265-01

Title: Nucleus Accumbens Core and Shell Analysis using Deep Brain Stimulation through functional Magnetic Resonance Image in rat

Authors: *H.-J. KIM[1], R. S. CLEMENT[2], R. BAGWELL[2], N. N. TIRKO[2], Y.-Y. I. SHIH[1], S.-H. LEE[1];
[1]Univ. of North Carolina At Chapel Hill, Chapel Hill, NC; [2]Actuated Med. Inc, State College, PA

Abstract: NAc (Nucleus accumbens) is known as the backbone of the brain's reward circuit, which consists of substructures of the core and shell. In this study, deep brain stimulation (DBS) was used with simultaneous functional magnetic resonance imaging (fMRI) to directly map their functional connectivity circuits. After inserting vertically aligned 16-channel flexible electrodes to be positioned over the core and shell simultaneously, three 10 sec blocks of electrical stimulation pulses (65 Hz) were sequentially delivered to each channel during each session and analyzed by the general linear model (GLM). (Fig. 1A-E). As a result, stimulation of the shell region of NAc showed strong BOLD signal elevation within unilateral medial prefrontal cortex (mPFC), anterior cingulate, and amygdala, while stimulation of core region showed strong BOLD signal elevation of bilateral mPFC and orbital prefrontal cortex regions. (Fig 1F, n=15) The activity map was parceled with a data-driven functional atlas obtained through our other study and subsequently clustered into 3 modules, the mPFC circuit, amygdaloid circuit, and default mode network (DMN). The data was time-locked at stimulus onset to investigate the dynamics of network-level interactions (Fig 1G-I). The results present that the stimulation of the shell induced a significant and immediate increase of BOLD signal in the Amygdala compared to the core, suggesting strong connectivity between structures. Interestingly, the stimulation of the NAc core derived the significant DMN activation in a delayed manner. We expect that the time-locked analysis of DBS-fMRI with multipoint stimuli can be a useful tool for future studying the dynamics of network interactions in mesoscale circuit regions with functionally distinct substructures.

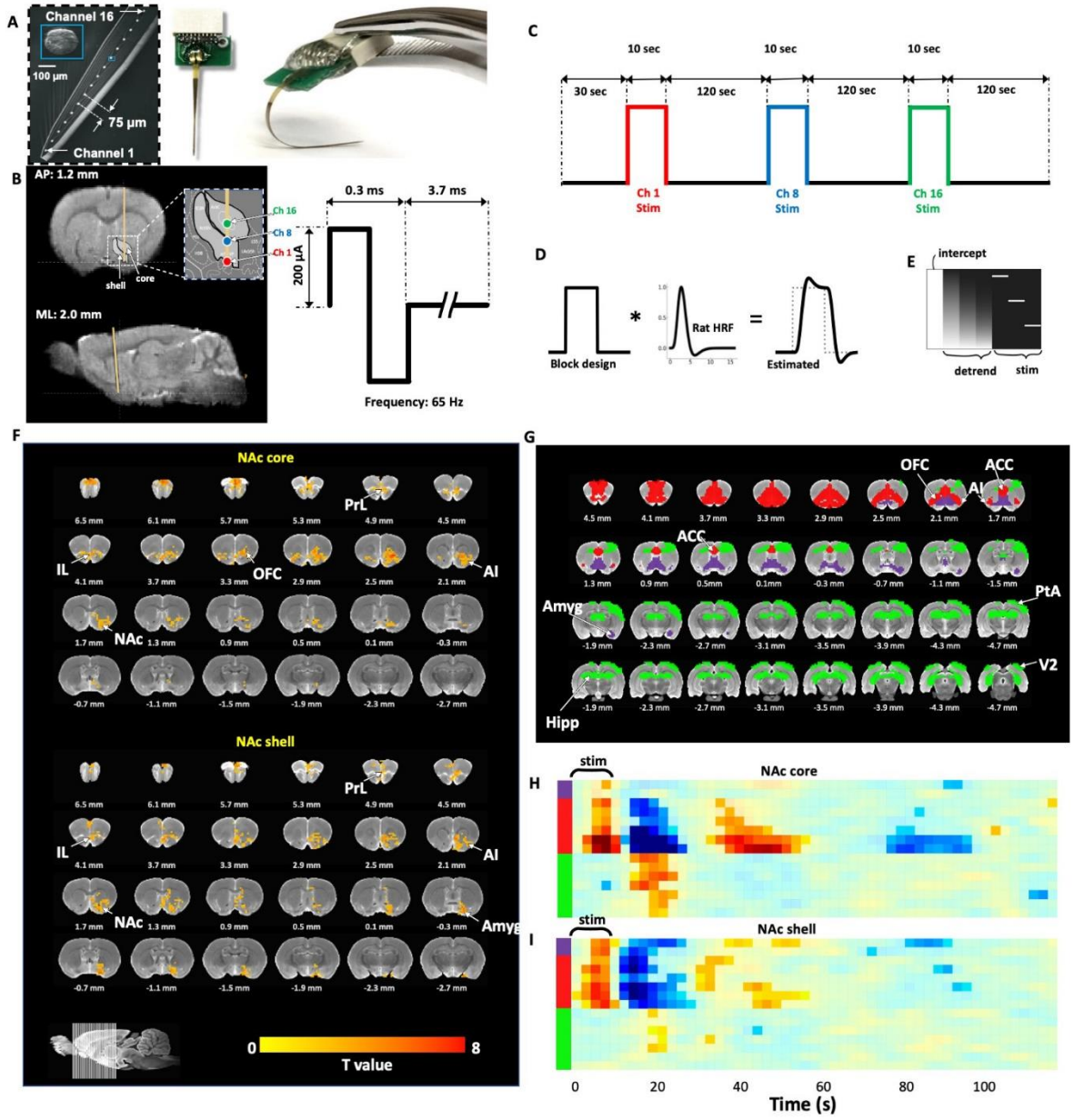


Figure 1. MR-compatible Multichannel neural probe implantation and stimulation paradigm (A-E), Activation Map (F), The network modulation effects NAc core and shell stimulation (G-I).
(A) An image presenting channel distance (75 um) and gross view of our MR-compatible multichannel neural probe, which have the flexibility. (B) Location of implantation and stimulation parameters and (C) stimulation paradigm. (D-E) Application of the GLM to fMRI data. Single-subject fMRI design matrix. Detrending 3rd order polynomial curve and yielding 1st level analysis with stimulation block. (F) NAc core and shell activation map. (G) Regions of interests (ROIs) based on DBS-fMRI activity map and Paxinos and Watson's rat brain atlas. Each network level mask representing prefrontal (red), amygdaloidal (purple), and default mode network (green) separately. (H-I) Heat map presenting group-level pattern of oscillation of each ROI after stimulation in NAc core and shell. (p< 0.05, FDR corrected) AI; anterior insula, Amyg; amygdala, ACC; agnesis of corpus callosum, PrL; prelimbic cortex, IL; infralimbic cortex, OFC; orbitofrontal cortex, Hipp; hippocampus, PtA; pericollicular tegmental area, V2; secondary visual cortex

Disclosures: H. Kim: None. **R.S. Clement:** None. **R. Bagwell:** None. **N.N. Tirko:** None. **Y.I. Shih:** None. **S. Lee:** None.

Poster

P939. Electrophysiology Methods: Electrode Arrays I

Location: Virtual Only

Time: Thursday, November 11, 2021, 1:45 PM - 2:45 PM

Program #/Poster #: P939.09

Topic: I.04. Physiological Methods

Title: Viability of single crystal monoclinic beta-gallium oxide as a substrate for intracortical electrode applications

Authors: *C. P. CAREY[1], M. H. KIM[1], B. KHAREL[1], J. LEE[1,2,3];
[1]Physics and Astronomy, [2]Neurosci., West Virginia Univ., Morgantown, WV; [3]WVU Rockefeller Neurosci. Inst., Morgantown, WV

Abstract: The substrate materials used for intracortical electrode devices are most commonly traditional semiconductor, predominantly silicon due to its mature microfabrication technology. When considering additional optical functions, opaque silicon requires integration of transparent structures such as glass or polymers. However, the wide bandgap materials possess both properties and thus have been introduced for neural applications with possible optical advantages. Recently, new advances in materials research are bringing to light more possibilities for substrate materials. In this study, we investigated the viability of a new optically transparent, electrically conductive substrate, single crystal monoclinic beta-gallium oxide (beta-Ga_2O_3), for the application of neural recording devices. The study showed that gallium oxide did not significantly degrade physically in artificial cerebrospinal fluid for a 14-week period at a temperature of 57 ^{O}C (equivalent to 56 weeks in body temperature). The impedance amplitude at 1 kHz fell within a range of 100 - 250 kΩ (vs. platinum wire counter electrode) for devices with an exposed area of 1960 μm^2. There were no changes in impedance spectra or 1 kHz impedance amplitudes indicative of degradation in the surface electrical properties of the substrate after a period of 45 days submerged in phosphate buffered saline at a temperature of 57 ^{O}C (equivalent to 180 days at body temperature). The devices were capable of clean recordings of a simulated action potential. These findings show that gallium oxide is an attractive candidate as a new substrate for intracortical electrode devices, namely for optical neural stimulation and imaging, as well as many other implantable devices.

Disclosures: C.P. Carey: None. **M.H. Kim:** None. **B. Kharel:** None. **J. Lee:** None.

Poster

P071. Development of Sensory Systems II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P071.01

Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: NIH Grant EY12716
NIH Grant EY022730
NIH Grant NS106244

Title: Synchrony filtering by electrical synapses in developing thalamus

Authors: *R. A. TIKIDJI-HAMBURYAN[1], G. I. GOVINDAIAH[2], W. GUIDO[2], M. T. COLONNESE[1];
[1]Pharmacol. and Physiol., The George Washington Univ. Sch. of Med., Washington, DC; [2]Dept. of Anatom. Sci. and Neurobio., Univ. of Louisville, Louisville, KY

Abstract: Before vision the retina produces spontaneous activity waves that help establish receptive field properties throughout the visual neuraxis. The circuit mechanisms and learning rules for this development are poorly understood. Retinal-waves correlate nearby ganglion cells on the order of 100ms, which is theoretically sufficient to drive topographic refinement through burst-time-dependent plasticity, but unlikely to be sufficient for further receptive field properties such as orientation selectivity. Recently Colonnese et al 2017 showed that despite poor retinal synchronization, nearby neurons in the dorsal lateral geniculate nucleus (dLGN) are synchronized on order of 10ms, which is critical to induce synaptic potentiation in the cortex. Here we use detailed cellular and network modeling to examine the potential role of electrical coupling between dLGN neurons (known to occur transiently during development) as a mechanism for this fast synchronization. Neuron intrinsic dynamics are critical for electrical coupling. Therefore, we used multiobjective optimization methods to obtain conductance-based biophysical models of dLGN relay neurons, and then distribute them into a heterogeneous population. Driving this population with RGC spikes recorded ex-vivo, showed that even 30% coupling probability induces short-latency synchronization of firing between dLGN neurons and improves localization of the wave propagations. Using a simplified model of two coupled neurons, we find that this synchronization is a result of NMDA-dominated synaptic currents, typical for development and sensitive to depolarization through electrical coupling, which induces a requirement for firing within ~100 - 200ms for a coupled postsynaptic neuron. However, this window increases presynaptic burst duration, suggesting synchrony filteringering in dLGN circuit. We explain this phenomenon using the analysis of the simplified model dynamics. Overall our model suggests that electrical coupling between thalamic relay neurons transforms low-temporally correlated firing of RGC into tightly synchronized thalamic activity necessary to refine visual sub-circuits for on/off responses and orientation selectivity.

Disclosures: R.A. Tikidji-Hamburyan: None. **G.I. Govindaiah:** None. **W. Guido:** None. **M.T. Colonnese:** None.

Poster

P071. Development of Sensory Systems II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P071.02

Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Title: Non-canonical dopaminergic neurons drive intraretinal vascular development

Authors: *J. H. LIANG, D. JIANG, M. A. SAMUEL;
Baylor Col. of Med., Houston, TX

Abstract: Retinal neuron function instructs and depends on the correct targeting of each of its three laminar-restricted vascular layers, in which defects in vascular organization are associated with a range of visual diseases. These vascular layers begin to emerge in the first postnatal week. The superficial layer forms first, then vessels abruptly grow outward to form the deepest vascular plexus, followed by inward growth to generate the intermediate layer. The neuronal cues that halt superficial layer growth and temporally and spatially instruct the transition to deep vessel growth remain unclear. Through previous findings, we posit that the neuron-derived signaling molecule, dopamine (DA), may be a critical player in the intraretinal vessel development pathway. Using pharmacological manipulation and tyrosine hydroxylase (TH) overexpression, we show that high levels of dopamine inhibit inner retina vessel development, with significant decrease in vessel branching. To identify the cellular source of dopamine responsible for vascular regulation, we employed transgenic mouse models with conditional deletion of TH from select subtypes of retinal neurons. We discovered that removing TH from canonical dopamine-synthesizing amacrine cells did not impact vessel patterning nor alter retina dopamine levels. In parallel, we have observed that tyrosine hydroxylase expression was unexpectedly present in retinal ganglion cell (RGC) layer neurons during the first postnatal week. Removing TH from RGCs or increasing the numbers of TH expressing RGC layer neurons markedly altered deep layer vessel emergence. These are pivotal findings that suggest there is another non-canonical cellular source of dopamine in the retina, other than dopaminergic amacrine cells, critical for intraretinal vasculature formation. From these results, we hypothesize that dopamine synthesis from the RGC layer initiates the proper vessel development of the superficial layer, allowing for subsequent transition and patterning of the deep retinal vessels.

Disclosures: J.H. Liang: None. **D. Jiang:** None. **M.A. Samuel:** None.

Poster

P071. Development of Sensory Systems II

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Program #/Poster #: P071.03

Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: DP2 MH125812
NARSAD Young Investigator Grant

Title: Subsynaptic refinement of neurotransmission machinery underlies activity-dependent eye-specific competition

Authors: *C. ZHANG[1], C. M. SPEER[2];
[1]Univ. of Maryland, Col. Park, College Park, MD; [2]Biol., Univ. of Maryland, College Park, MD

Abstract: The development of eye-specific retinogeniculate connections is a classic model for studying activity-dependent synaptic competition and refinement. In the mouse, axons from the left and right eyes overlap in the dorsal lateral geniculate nucleus (dLGN) and undergo activity-dependent competition during the first postnatal week. Investigating the synaptic basis of such refinement is challenging due to the limited resolution of conventional optical microscopy and difficulties in identifying immature retinogeniculate synapses with electron microscopy. Here we used volumetric STochastic Optical Reconstruction Microscopy (STORM), a single-molecule localization super-resolution imaging technique, together with eye-specific anterograde tracing and synaptic immunolabeling to measure the nanoscale structural and molecular refinement of eye-specific retinogeniculate synapses from P2-P8 in the mouse. By measuring synaptic densities across development, we found that eye-specific synaptic competition is delayed until after P2, reflecting the late arrival of ipsilateral axons relative to contralateral inputs. Eye-specific synapses show a developmental increase in presynaptic active zone (AZ) and vesicle protein abundance with no change in postsynaptic density (PSD) protein expression. These changes are further reflected in a development increase in the total number and spatial compaction of subsynaptic domains of presynaptic AZ and vesicular proteins with no change in PSD organization. To examine the role of neural activity in eye-specific competition, we analyzed synaptic refinement in a transgenic mutant model with disrupted cholinergic retinal waves and arrested eye-specific segregation. Abnormal retinal wave activity blocked retinogeniculate synaptogenesis and reduced presynaptic protein abundance with no impact on PSD protein expression in individual synapses. Activity-mutant presynaptic terminals had fewer subsynaptic domains (SSDs) and showed no spatial refinement compared to controls. Concurrently, the presynaptic readily releasable pool showed developmental and activity-dependent increases during normal eye-specific refinement. These results highlight a role for spontaneous retinal activity in regulating presynaptic refinement during eye-specific competition. Disruption of retinal waves prevents synaptogenesis and causes axon elaboration consistent with a synaptotropic hypothesis of axon branch stabilization. These data highlight the power of super-resolution microscopy for investigating subsynaptic molecular organization and plasticity in developing circuits.

Disclosures: C. Zhang: None. **C.M. Speer:** None.

Poster

P071. Development of Sensory Systems II

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Program #/Poster #: P071.04

Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: NIH P30 Grant EY007003
Matilda E. Ziegler Research Award
Grant from Research to Prevent Blindness

Title: Dominant negative mutations in the small GTPase Arl3 lead to rod nuclear migration defects

Authors: *A. M. TRAVIS, S. MANOCHA, J. R. WILLER, J. N. PEARRING;
Univ. of Michigan, Ann Arbor, Ann Arbor, MI

Abstract: Human mutations in the small GTPase Arl3 cause a variety of inherited retinal dystrophies, including rod-cone dystrophy and retinitis pigmentosa, where blindness is caused by the degeneration of specialized neurons in the retina. These mutations are generally believed to destabilize Arl3 or impair its interactions with binding partners; however, the underlying pathobiology has yet to be studied *in vivo*. Here, we use *in vivo* mouse models as well as *in vitro* biochemistry and cell-based experiments to understand the cellular consequences dominant Arl3 mutations have in rod photoreceptors. We exogenously expressed FLAG-tagged Arl3 constructs representing either characterized GTPase-cycle mutants or retinal degeneration-causing mutants in wild type mouse rod photoreceptors via *in vivo* electroporation. Visualization of Arl3-FLAG in immunostained retinal cross-sections revealed that dominant mutations in Arl3 cause rod photoreceptor nuclei to be abnormally distributed within their nuclear layer and even sometimes mislocalized to an incorrect nuclear layer, a phenotype previously linked to retinal degeneration. *In vitro* biochemistry and cell-based assays were performed in transfected AD293 cells to characterize GTP- and effector-binding of these Arl3 mutants. We show that the dominant human Y90C mutation results in increased binding to the guanine exchange factor, Arl13B, as well as aberrant Arl3 activity in cells. We further determine that the Arl13B sequestration by Arl3-Y90C rather than the aberrant activation of the Arl3-Y90C causes the *in vivo* nuclear migration defect. These findings both expand our understanding of how small GTPases function normally and abnormally in cells and provide a model to study the nuclear component of neuronal migration.

Disclosures: A.M. Travis: None. **S. Manocha:** None. **J.R. Willer:** None. **J.N. Pearring:** None.

Poster

P071. Development of Sensory Systems II

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Topic: A.08. Development of Motor, Sensory, and Limbic Systems

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Title: Early and late stages in the development of the avian visual tecto-isthmo-fugal pathway

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Abstract: The tectofugal pathway, a highly conserved retino-midbrain-fugal visual system, is the main visual pathway in birds and many mammals. In amniotes the tecto-fugal projection arises from the tectal ganglion cells (TGCs) which possesses a broad and large dendritic field and specialized “bottle brush" dendritic endings (BBE) that distribute in specific superficial tectal layers. In birds these endings receive synaptic contact from retinal fibers of the contralateral eye and from columnar, "paintbrush"-like axons terminals from the nucleus isthmi parvocellularis (Ipc). The Ipc nucleus, part of the highly conserved isthmo-tectal network, exerts a strong control on the propagation of visual activity from the TGCs to higher visual areas. TGCs axons projects bilaterally to the nucleus rotundus (Rt), which in turn, project to the visual dorsal ventricular ridge (DVR) in the pallium. This pallial region is made up of 3 layers interconnected in a columnar/recurrent arrangement: entopallium (E), in receipt of afferents from the Rt, intermediate nidopallium, and mesopallium. In this work we sought to describe the ontogenetic establishment and consolidation of the avian tecto-isthmo-fugal pathway. Neurotracing “ex-vivo” experiments revealed a highly organized Rt-E projection as early as E8, and columnarly organized axons, reciprocally and homotopically connected through the DVR layers, from E12 onwards. Immunohistochemical assays suggest that both system of projection probably emerges in conjunction at earlier stages, between E6 and E8. On the contrary, Ipc axons exhibit a mature paintbrush morphology late in development, only at about E18. Since dendrites of TGCs reach the retinorecipient layers around E16, and mature endings are detected only at E18, a stage when an essentially mature pattern of retinotectal connectivity is described, the development of the avian tectofugal pathway seems to have two main phases. During the first phase, the tecto-thalamo-pallial circuitry and the intrapallial arrangement is established, and subsequently, during a second phase, a mature retino-istmo-tectal projection is attained. This suggests that the development of the thalamic and pallial stages of the tectofugal pathway largely proceeds independently of retinal inputs, and that transmission of retinal activity through this pathway, being spontaneous or visually-elicited, would only be possible from E16-E18 onwards.

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Poster

P071. Development of Sensory Systems II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P071.06

Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: NIDCD R01DC008860
The family of William Ziegler III

Title: Efferent feedback controls bilateral auditory spontaneous activity

Authors: *Y. WANG[1], M. SANGHVI[2], A. GRIBIZIS[3], Y. ZHANG[1], L. SONG[4], B. J. MORLEY[5], D. BARSON[6], J. SANTOS-SACCHI[8], D. NAVARATNAM[7], M. C. CRAIR[3]; [1]Dept. of Neurosci., [2]Dept. of Neuroscience,, Yale Univ. Sch. of Med., New Haven, CT; [3]Dept. of Neurosci., Yale Univ., New Haven, CT; [4]Shanghai Ninth People's Hosp., Shanghai Jiao Tong Univ. Sch. of Med., Shanghai, China; [5]Boys Town Natl. Res. Hosp, Omaha, NE; [7]Dept. of Surgery (Otolaryngology), [6]Yale Sch. of Med., New Haven, CT; [8]Yale Univ. Sch. Med., New Haven, CT

Abstract: In the developing auditory system, spontaneous activity generated in the cochleae propagates into the central nervous system to promote circuit formation. The effects of peripheral firing patterns on spontaneous activity in the central auditory system are not well understood. Here, we describe wide-spread bilateral coupling of spontaneous activity that coincides with the period of transient efferent modulation of inner hair cells from the brainstem medial olivocochlear system. Knocking out α9/α10 nicotinic acetylcholine receptors, a requisite part of the efferent pathway, profoundly reduces bilateral correlations. Pharmacological and chemogenetic experiments confirm that the efferent system is necessary for normal bilateral coupling. Moreover, auditory sensitivity at hearing onset is reduced in the absence of pre-hearing efferent modulation. Together, these results demonstrate how afferent and efferent pathways collectively shape spontaneous activity patterns and reveal the important role of efferents in coordinating bilateral spontaneous activity and the emergence of functional responses during the prehearing period.

Disclosures: Y. Wang: None. **M. Sanghvi:** None. **A. Gribizis:** None. **Y. Zhang:** None. **L. Song:** None. **B.J. Morley:** None. **D. Barson:** None. **J. Santos-Sacchi:** None. **D. Navaratnam:** None. **M.C. Crair:** None.

Poster

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Topic: A.08. Development of Motor, Sensory, and Limbic Systems

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Title: Oncomodulin Alters Spontaneous Calcium Activity in Outer Hair Cells via Purinergic Signaling During Development

Authors: Y. YANG, K. MURTHA, L. CLIMER, A. HORNAK, *D. D. SIMMONS;
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Abstract: In the mammalian cochlea, the role of spontaneous Ca^{2+} activity in the outer hair cells (OHCs) is critical to postnatal development and can be modulated by ATP-dependent activation of purinergic receptors. The tight regulation of Ca^{2+} in OHCs is also essential for both development and mechanotransduction. A plethora of Ca^{2+}-binding proteins (CaBPs) are involved in maintaining Ca^{2+} homeostasis. Oncomodulin (OCM, β-parvalbumin), an EF-hand CaBP, is expressed preferentially in OHCs, and is the only CaBP known to cause progressive hearing loss after genetic deletion. We hypothesize, during the critical period of development, the absence of OCM alters the spontaneous Ca^{2+} activity in OHCs before the onset of hearing.Using *Ocm* wild-type (WT) and *Ocm* knockout (KO) mice at postnatal day 2 (P2) expressing a genetically encoded tissue-specific Ca^{2+} sensor (*Atoh1*-GCaMP6s), we observed both *Ocm*-WT and *Ocm*-KO OHCs exhibited spontaneous Ca^{2+} activity. The fractional changes of fluorescence intensity ($\Delta F/F_0$) of activated Deiters' cells (DCs) and all OHCs in a single field of view were calculated. We found Ca^{2+} activity initiated from the greater epithelium region (GER), temporally synchronized Ca^{2+} activity in both DCs and OHCs. The average correlation coefficient of DCs and OHCs showed a positive relationship with the longitudinal extension of Ca^{2+} activity. This correlation had a linear regression coefficient significantly different from zero ($P < 0.0001$, *f*-test) in both *Ocm*-WT and *Ocm*-KO mice. Additionally, *Ocm*-KO OHCs exhibited greater synchronization when the lontidudinal extension of Ca^{2+} activity in the GER increased (slope rate, 3.70×10^{-3} μm^{-1}) compared to *Ocm*-WT OHCs (slope rate, 3.00×10^{-3} μm^{-1}). In *Ocm*-WT spirals, all Ca^{2+} wave extensions over 53 μm showed significantly synchronized Ca^{2+} signals with OHCs (25 waves analyzed) compared to the background. In *Ocm*-KO OHCs, this extension threshold is decreased to 33 μm. *Ocm*-KO OHCs showed significantly increased average maximum $\Delta F/F_0$ compared to *Ocm*-WT OHCs during Ca^{2+} events in the GER ($P<0.01$, t-test). The spontaneous Ca^{2+} activities were abolished in either Ca^{2+} - free solution or the medium applied with 250 μM nifedipine. The purinergic receptor subtype P2X2 is proposed to mediate spontaneous Ca^{2+} waves in the cochlea. Using qRT-PCR and immunocytochemistry, the P2X2 expression was upregulated in *Ocm*-KO spirals. These data indicate that *Ocm*-KO spirals exhibit a high degree of spontaneous Ca^{2+} signaling at early stages of development, and this activity may be due to purinergic receptor expression. Whether this higher activity leads to deleterious consequences in developing OHCs is not known at present.

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Poster

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Program #/Poster #: P071.08

Topic: A.08. Development of Motor, Sensory, and Limbic Systems

Support: Czech Science Foundation Grant 20-06927S

Title: Conditional deletion of ISLET1 affects the formation of the spiral ganglion, auditory neurons projections and auditory information processing

Authors: *K. PYSANENKO[1], I. FILOVA[2], M. TAVAKOLI[2], M. DVORAKOVA[2], S. VOCHYANOVA[2], R. BOHUSLAVOVA[2], B. FRITZSCH[3], G. PAVLINKOVA[2], J. SYKA[1]; [1]Inst. of Exptl. Med. CAS, Prague, Czech Republic; [2]Inst. of Biotech. CAS, Vestec, Czech Republic; [3]Dept. of Otolaryngology, Univ. of Iowa, Iowa City, IA

Abstract: The development of inner ear sensory neurons is governed by a number of transcription factors including *ISLET1* (*Isl1*), a LIM homeodomain (LIM-HD) transcription factor. We have developed a novel conditional deletion model of *Isl1* (*Isl1cKO*). Using Neurod1Cre, we specifically eliminated *Isl1* in spiral ganglion neurons without affecting the development of sensory epithelium. The morphological analyses showed that primary auditory neurons, deprived of the transcription factor *Isl1*, form disorganized peripheral projections and dramatically reduced central projections. Since *Isl1cKO* mice are viable, we evaluated how morphological changes in the cochlea affect the properties of auditory processing. With the aim to evaluate physiological functions, we measured the distortion product otoacoustic emissions (DPOAE) and auditory brainstem responses (ABRs). In addition, we evaluated the responses to acoustical stimuli by recording single and multiple unit activity in the inferior colliculus and studied the strength of the auditory startle responses, (ASRs) as well as the efficiency of the prepulse inhibition of ASR. The morphological changes were associated with significant hearing impairment in adult mice. In comparison with controls, the DPOAEs in *Isl1cKO* were absent or significantly decreased for low and middle frequencies but remained unchanged at the high frequency band. ABR thresholds were increased in a similar manner. Responses of inferior colliculus neurons in the *Isl1cKO* mice were characterized by a reduced tonotopic range and increased excitatory thresholds. There were various shapes of tuning curves present, from the simple control-like to wide or multi-peaked, reflecting the tuning impairment of *Isl1cKO* mutants. Acoustic behavioural tests confirmed altered responsiveness to suprathreshold acoustic stimuli especially in the low frequency band. These findings demonstrate that genetically altered properties of neurons in the cochlea may have complex consequences for acoustic signal processing and auditory behavioral performances underlying the *Isl1* deletion.

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Poster

P130. Modulation of Neuronal Firing Properties

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Program #/Poster #: P130.01

Topic: B.08. Intrinsic Membrane Properties

Support: NIH Grant R01DA047870
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Title: Intrinsic and training-dependent gradients of timescales of neural dynamics within the primate prefrontal cortex

Authors: *E. TREPKA[1], M. SPITMAAN[1], X.-L. QI[2], C. CONSTANTINIDIS[3], A. SOLTANI[1]; [1]Dept. of Psychological & Brain Sci., Dartmouth Col., Hanover, NH; [2]Dept. of Neurobio. and Anat., Wake Forest Univ. Sch. of Med., Winston Salem, NC; [3]Dept. of Biomed. Engin., Vanderbilt Univ., Nashville, TN

Abstract: Cortical neurons exhibit multiple timescales related to dynamics of spontaneous fluctuations (intrinsic timescales), response to task events (seasonal timescales), and temporal integration of task-relevant signals such as choice and reward outcome. These timescales are uncorrelated within individual neurons but increase systematically across the cortical hierarchy, e.g., from parietal to prefrontal and cingulate cortex. It is currently unknown whether these timescales depend on extensive training in a specific task and/or are an inherent property of cortical computations and whether more fine-grained hierarchies of timescales exist within specific cortical areas. To address these questions, we analyzed single-cell neural recordings within subregions of the prefrontal cortex (PFC) of macaques before and after training on a working-memory (spatial match/nonmatch) task (N = 6 animals, 2,975 neurons). We found that after training, seasonal timescales increased across the prefrontal hierarchy from posterior to anterior subregions, within both dorsal and ventral PFC. This effect appeared to be the result of task training; no such hierarchy was present in the same areas, prior to training. On the other hand, intrinsic timescales decreased along the posterior-anterior axis and this effect was present even before training. Training was accompanied by an increase in the fractions of neurons selective to visual stimuli in more anterior regions. Interestingly, these changes in overall selectivity were accompanied by a decrease in the percentage of neurons that exhibited timescales tied to the timing of the appearance of the visual stimuli in the trial and increased percentages of neurons that exhibited intrinsic timescales, most significantly in anterior regions. Consistent with results from prior studies, in other tasks, timescale hierarchies in PFC were

uncorrelated within individual neurons and independent of selectivity to task-relevant signals.Together, our results provide evidence that neuronal firing is governed by both inherent and learning-shaped, fine-grained hierarchies of timescales within the PFC that can be further shaped by training in a task, pointing to flexibility of canonical computations within the cortex.

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Poster

P130. Modulation of Neuronal Firing Properties

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Program #/Poster #: P130.02

Topic: B.08. Intrinsic Membrane Properties

Support: NS077675

Title: Differential axon initial segment morphology and physiology between type A and type B layer 5 pyramidal neurons

Authors: *A. HARRIS, S. D. BENUSA, J. L. DUPREE, K. M. JACOBS;
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Abstract: Within neocortical layer 5, pyramidal neurons can be differentiated into at least two major subclasses on the basis of their projections to the brainstem and thalamus (type A), or to the contralateral cortex and basal ganglia (type B). These two types can be discerned on the basis of surrogate measures of physiology and morphology; in particular, type A manifests a prominent I_h and possesses a wider apical dendrite compared to type B. Because the axon initial segment (AIS) is a crucial locus for control of neuronal excitability, we hypothesized that there may exist functional and/or morphological differences between type A and type B pyramidal neurons.
To investigate this hypothesis, acute brain slices were prepared from male and female C57BL/6NHsd mice, and high-resolution action potentials (AP) and standard IVs were recorded from randomly selected layer 5 pyramidal neurons, which were filled with biocytin and stained post hoc for the AIS scaffolding protein ankyrinG. Layer 5 pyramidal neurons separated into two non-overlapping clusters on the basis of the sum of their sag and their depolarizing afterpotential, calculated as previously described. These two groups were considered type A and type B for further analysis (*N = 42 type A cells; N = 24 type B cells*). Results are reported as mean ±SEM; t-tests were used to compare between neuronal types, with $p<0.05$ considered significant.
Type A neurons manifested a significantly higher AIS- and soma-regional voltage acceleration during the AP upstroke as measured by the second derivative of the AP (*in $mV^2/msec^2$ for type A vs B: AIS peak 2051 ±78 vs 1512 ±102; soma peak 2601 ±102 vs 1855 ±124*). In addition, type A

neurons had a higher frequency-current slope (*165.8 ±8.1 vs 139.4 ± 10.2 Hz/nA*), lower rheobase (*38.8 ±5.5 vs 60.8 ±7.4 pA*), a more hyperpolarized AP threshold (*-48.5 ±0.5 vs -44.8 ±0.6 mV*), and a higher maximum firing frequency (*58.0 ±2.4 vs 41.4 ±3.0 Hz*). Morphologically, type A neurons had a wider apical dendrite (*3.4 ±0.2 vs 2.5 ±0.3 µm*). Intriguingly, the AIS start position as measured by ankyrinG and $Na_V1.6$ immunostaining was located more proximally in type A neurons compared to type B (*1.9 ±0.4 vs 4.2 ±1.1 µm from soma, ankyrinG; 3.6 ±0.4 vs 5.5 ±1.0 µm from soma, $Na_V1.6$).* Taken together, these results suggest that type A neurons are more intrinsically excitable and may possess a higher density of sodium channels at the AIS and soma compared to type B neurons. This functional specialization may exist to support the fidelity of AP transmission to the comparatively longer projection distances type A neurons must cover.

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Poster

P130. Modulation of Neuronal Firing Properties

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Topic: B.08. Intrinsic Membrane Properties

Support: PAPIIT-DGAPA UNAM IN 102420
Conacyt CB A1-S-10610

Title: Slow-fast molecular dynamics explain tunable correlations between homeostatically regulated maximal conductances

Authors: *M. ORDORICA[1], J. P. GOLOWASCH[2], A. FRANCI[3];
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Abstract: Neural systems are able to maintain their functions despite the constant changes in tasks and activities and the external disturbances they are subjected to. Recent computational models ([2, 1]) explain this resilience using integral controllers to monitor the intracellular calcium concentration with the premise that, if intracellular calcium is kept at a target value, the neuron will preserve its electrical properties despite disturbances and changes. While these computational models explain the stability of neuronal activity, they impose unrealistically strong correlations between all pairs of regulated conductances. Research conducted in the mouse hippocampal granule cells shows that only specific conductances are strongly correlated. In contrast, other pairs show a low or null correlation coefficient [3]. To explain these observations, we present a mathematical model that includes a molecular homeostatic regulation network with a fast and a slow dynamical component. We show that by suitably designing the slow dynamics of the homeostatic regulation network, it is possible to flexibly tune the

correlation strength between all conductance pairs. In other words, by allowing rich dynamical interactions between molecular regulatory variables, it is possible to capture the flexible degree of correlation observed in experiments. Our results reveal the importance of including sufficiently structured homeostatic regulation networks in neural homeostasis models.

References

[1] Franci, A., O'Leary, T., Golowasch, J. (2020). Positive Dynamical Networks in Neuronal Regulation: How Tunable Variability Coexists With Robustness. IEEE Control Systems Letters, 4(4), 946-951.

[2] O'Leary, T., Williams, A. H., Franci, A., Marder, E. (2014). Cell types, network homeostasis, and pathological compensation from a biologically plausible ion channel expression model. Neuron, 82(4), 809-821.

[3] Tran, T., Unal, C. T., Severin, D., Zaborszky, L., Rotstein, H. G., Kirkwood, A., Golowasch, J. (2019). Ionic current correlations are ubiquitous across phyla. Scientific reports, 9(1), 1-9.

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Poster

P130. Modulation of Neuronal Firing Properties

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Program #/Poster #: P130.04

Topic: B.08. Intrinsic Membrane Properties

Support: F31NS108579
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Title: Restoring prefrontal hypoexcitability rescues social behavior deficits in a mouse model of late-stage frontotemporal dementia

Authors: *H. PHILLIPS[1], H. DAI[1], S. CHOI[2], L. DALY[2], L. PETRUCELLI[3], F.-B. GAO[2], W.-D. YAO[1];
[1]Psychiatry and Neurosci., SUNY Upstate Med. Univ., Syracuse, NY; [2]Univ. of Massachussetts Med. Sch., Worcester, MA; [3]Dept. of Neurosci., Mayo Clin., Jacksonville, FL

Abstract: Frontotemporal dementia (FTD) is associated with progressive atrophy of the frontal and temporal lobes. It is the second most common type of dementia after Alzheimer's Disease, and there is no cure. The major subtype of FTD is behavioral variant FTD (bvFTD), characterized by changes in personality, inappropriate social conduct, loss of empathy, anxiety, compulsive behaviors, overeating and disinhibition. The underlying neural mechanisms are largely unknown. A GGGGCC (G_4C_2) hexanucleotide repeat expansion (HRE) in the *C9ORF72* gene is the most common genetic risk for FTD and amyotrophic lateral sclerosis. We have developed an AAV-based somatic transgenic mouse model that expresses 66-G_4C_2 HREs

throughout their central nervous system and recapitulates all major behavioral deficits of FTD. AAV-$(G_4C_2)_{66}$ mice develop impaired sociability, increased anxiety, risk taking-like behavior and increased food intake at approximately 12 months of age. *Ex vivo* whole-cell patch-clamp electrophysiology on layer V pyramidal neurons of medial prefrontal cortex (mPFC) slices showed a significant decrease in neuronal excitability characterized by a profound loss of action potential firing, increased rheobase, and reduced membrane input resistance. Significantly, restoring membrane excitability in the mPFC by *in vivo* chemogenetics effectively rescued social behavior deficits in aged mutant mice. These results demonstrate that prefrontal hypoexcitability mediates FTD-associated sociability impairments, which importantly are rescuable even at a late disease stage when significant neural degeneration has occurred. Thus, targeted restoration of prefrontal cortex excitability could serve as a promising therapy for treating late-stage *C9ORF72*-associated FTD.

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Poster

P130. Modulation of Neuronal Firing Properties

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Topic: B.08. Intrinsic Membrane Properties

Support: DA032283
MH106489
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Title: Hypoactivity of the infralimbic cortex-nucleus accumbens circuit underlies cocaine conditioned place preference

Authors: *A. M. KOBEISSI, H. L. PHILLIPS, X. WU, W. YAO;
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Abstract: Drug addiction is characterized by chronic and compulsive drug use and relapse despite negative consequences. A hallmark of addiction is loss of impulse control, and treatments seeking to restore this cognitive function may promote recovery. The prefrontal cortex (PFC) exerts top-down inhibitory control of subcortical nucleus accumbens (NAc) functions and goal-directed behaviors such as motivation, choice, and reward. In a current view, addictive drugs impair the PFC, leading to hypofrontality and consequential loss of inhibitory control over drug seeking behavior as well as the ability to extinct drug use memories. However, the underlying neural mechanisms remain poorly understood. Combining cocaine conditioned place preference

(CPP) behavior, retrograde circuit tracing, slice patch-clamp electrophysiology, and in vivo chemogenetics in mice, we examined changes in neuronal intrinsic excitability of layer V (LV) prelimbic cortex (PrL) and infralimbic (IL) pyramidal neurons in the PFC that project to the NAc core (NAcC) and shell (NAcSh), respectively, following chronic cocaine administration during CPP. We found that cocaine CPP induced a profound decrease of neuronal excitability of IL-NAcSh projection neurons, characterized by significantly reduced action potential firing frequency, decreased membrane input resistance, increased rheobase, and enhanced afterhyperpolarizations. This cocaine-induced IL-NAcSh circuit hypoactivity lasted for at least two weeks following cocaine withdrawal. In contrast, the intrinsic excitability of PrL-NAcC projection neurons also became significantly decreased after 1-day withdrawal from cocaine CPP but recovered after 15 days of cocaine withdrawal. To test if restoration of neuronal excitability attenuates cocaine CPP behavior, we delivered bilateral microinjections of AAV2-hSyn-DIO-hm3dq-mCherry into the IL and rAAV2-EF1a-mCherry-IRES-wga-cre into the NAcSh to selectively enhance the activity of IL-NAcSh pathway following intraperitoneal administration of clozapine N-oxide (CNO). Mice administered with CNO did not spend more time in the drug paired environment compared to time spent in the saline paired environment on both withdrawal day 1 and 15, suggesting that restoration of the activity of IL-NAcSh circuit is involved in place preference induced by cocaine. Our results demonstrate that the prefrontal IL-NAcSh pathway is more preferentially and sustainably impaired by chronic cocaine administration and this top-down PFC pathway exerts profound control on cocaine reward behavior.

Disclosures: A.M. Kobeissi: None. **H.L. Phillips:** None. **X. Wu:** None. **W. Yao:** None.

Poster

P130. Modulation of Neuronal Firing Properties

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Program #/Poster #: P130.06

Topic: B.08. Intrinsic Membrane Properties

Support: SC2GM122646

Title: Electrophysiological characterization of VTA-projecting BNST neurons in mice

Authors: *Y. MIURA[1,2], M. SHANLEY[1,2], R. KARIM[1], A. K. FRIEDMAN[1];
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Abstract: The Bed Nucleus of Stria Terminalis (BNST) is involved in the regulation of a diverse range of behavioral states, including anxiety, reward processing, and appetite control. This diversity is achieved by a distinct mix of opposing neurons within the BNST, both excitatory (glutamatergic) and inhibitory (GABAergic) cell types which have multiple projecting

substrates. Recent mouse studies have utilized cell-type or projection-specific manipulations to identify these neurons' important role in behavioral modulation. Specifically, these studies demonstrate that optogenetic or chemogenic activation or inhibition of individual subpopulations frequently function to induce opposing behavioral outputs. However, the role of the functional electrophysiological diversity within these projections remains understudied.
The goal of the study is to characterize and explore the deeper role of the functional heterogeneity within the BNST's GABAergic subpopulation that projects to the Ventral Tegmental Area (VTA). This limbic connection between the BNST and VTA serves a critical role in integrating information regarding anxiety, modulated in part by corticotropin-releasing factor and reward signaling modulated by dopamine. Revealing the functional differences in electrophysiological properties in this projection will provide insight into circuit mechanisms of stress resilience.
This GABAergic subpopulation has been divided into type I, type II, and type III cells based on the individual electrophysiological properties in rats. However, electrophysiological properties of the VTA-projecting BNST neurons and their functional role are not fully characterized in mice. To this end, we used a combination of a retrograding adeno-associated virus with an EYFP tag (rAAV-hSyn-EYFP) injected into the VTA of 8-week-old C57Bl6/J male mice to identify and perform projection-specific electrophysiological characterization. Following recovery, we performed ex vivo whole-cell patch-clamp recordings to evaluate intrinsic and extrinsic differences which include: 1) resting membrane potential, 2) excitability, 3) voltage sag, 4) inter-spike interval, 5) latency to first spike, 6) Ih current, and 7) maximum firing rate. Utilizing an unbiased analysis of the individual parameters we have identified three functionally distinct VTA-projecting BNST neurons in mice, replicating rat studies. To further explore the functional heterogeneity with this subpopulation we will be exploring the differing inputs of these individual cell-types.

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Poster

P130. Modulation of Neuronal Firing Properties

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Program #/Poster #: P130.07

Topic: B.08. Intrinsic Membrane Properties

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Title: The properties of neurons in the lateral septum: physiology, morphology and pharmacology

Authors: *B. GURUNG[1], A. D. RANDALL[2], J. T. BROWN[1];
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Abstract: Glucagon-like peptide-1 receptor (GLP-1R) is an important G-protein coupled receptor in the periphery, which when activated stimulates the glucose-dependent release of insulin by the pancreatic β-cells to lower blood glucose. As such drugs that are agonists of this receptor are an approved class of drugs used for Type 2 diabetes mellitus (T2DM). T2DM and the neurodegenerative disease Alzheimer's disease (AD) share common pathophysiological characteristics such as insulin resistance and decreased glucose metabolism. The GLP-R agonist liraglutide is under a Phase IIb clinical trial (NCT01843075) in patients with mild AD dementia to examine the drug's effect on the hallmarks of the progression of the disease. Other agonists of this receptor have also shown improved spatial learning and memory in animal models of AD. GLP-1R activation in the brain is also associated with cell survival, inhibition of apoptosis, cell growth, synapse growth, repair and regeneration. However, to date the details of the effects this receptor system has at a neural circuit level in the brain is not well known. GLP-1R is expressed in many brain structures but it is most highly expressed in the lateral septum. Thus, our research will be based on this subcortical brain structure involved in a wide variety of functional roles such as food intake, stress and anxiety, reward and addiction, contextual memory and locomotion. Therefore, we aim to explore the effects of GLP-1R activation on neuronal activity in the lateral septum. However, in order to study the effects of GLP-1R activation, it is important to first characterise the baseline properties of the neurons in the lateral septum. To this, we have used a multi-electrode array approach combined with computational analysis to identify different potential sub-populations of neurons within the lateral septum. Our data also suggests that the anatomical distribution of these neuronal sub-populations based on physiology is heterogeneous. We also used whole patch clamp method to characterise the morphological and intrinsic properties of individual lateral septal neurons. We will then aim to explore whether different sub-populations respond differently to GLP-1R agonists. Consequently, our work will provide a basis for future works to study whether the effects of GLP-1R activation on neuronal activity is a result of direct or modulatory mechanism, and also how the regulation of neural circuits by this receptor system guides behaviour.

Disclosures: B. Gurung: None. **A.D. Randall:** None. **J.T. Brown:** None.

Poster

P130. Modulation of Neuronal Firing Properties

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P130.08

Topic: B.08. Intrinsic Membrane Properties

Support: NIH Grant 1UF1NS115821

Title: Intrinsic Properties of Basal Ganglia Projecting Neurons Reflect Specific Learned Features of Zebra Finch Song

Authors: *N. MEDINA, D. MARGOLIASH;
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Abstract: The study of plasticity in the intrinsic properties (IPs) of neurons is unveiling new mechanisms beyond synaptic plasticity that relates cellular plasticity, network activity, and learning. Previous results from adult zebra finches established a relationship between the IPs of forebrain neurons and learned singing behavior. Within the song control nucleus HVC, prior studies have reported the IPs of HVC basal-ganglia-projecting neurons (HVCx) are similar within individual zebra finches and different across birds. Those differences were predictive of the overall differences of the birds' songs. Additionally, HVCx IPs are developmentally regulated, such that IPs may become increasingly homogeneous during developmental song learning. Given these prior results, we are directly examining the role of song learning in regulating HVCx IPs. Our approach uses a counterbalanced design to raise sibling or unrelated birds to sing natural or modified songs, using both live tutors and instrumental learning. We assess the IPs of HVCx neurons in slice once birds reach adulthood, evaluating IPs in relation to the tutored song and the song eventually acquired. Preliminary results indicate that the song, not relatedness to the tutor, has the stronger effect on the IPs HVCx will express in adulthood. Examining the correlation between IPs and song features, we have also observed a relationship between timing features of song and the rebound excitation of HVCx neurons. HVCx neurons from birds who sing longer songs, which tend to include longer harmonic stacks, also tend to have a combination of IPs that reflect increased rebound excitation. This demonstrates an explicit link between neuronal IPs and specific features of singing behavior, implicates specific membrane channels (gh, gSK, gCa-T), and given that harmonic stacks are relatively static vocalizations, suggests a mechanism underlying HVCx neurons' ability to integrate over relatively long periods of song. These results constrain models of HVC and implicate the importance of including neuronal IPs in developing realistic network-level descriptions of neural circuits. They also provide further evidence that HVCx activity is not independent of song structure.

Disclosures: N. Medina: None. **D. Margoliash:** None.

Poster

P140. Networks

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P140.01

Topic: B.10. Epilepsy

Support: CA in Neuroendocrinología BUAP-CA-288
JMIH fellowship from CONACYT

Title: The reticular nucleus of the thalamus modulates the onset of the absence seizures on the myelin mutant taiep rat

Authors: *J. M. IBARRA-HERNÁNDEZ[1], C. CORTES[1], J. R. EGUIBAR[2];
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Abstract: The absence seizures are one type of epilepsy which is characterized by a sudden loss of consciousness and with characteristic spike-wave discharges (SWD) in the electroencephalogram. Absence seizures are due to a dysregulation in the activity of the gamma-aminobutyric acid receptor type A ($GABA_A$) in the thalamo-cortical network which are modulate by the reticular nucleus of the thalamus (RNT). The aim of this study was to analyze the role of $GABA_A$ receptors in the RNT on the SWD on adult male *taiep* rats. We used 6 months old *taiep* rats which were implanted with electrodes in the RNT, cerebral cortex, neck muscles and in the right eye orbit for simultaneous recordings. Gaboxadol was infuse at 7.5, 15 y 30 µg/µL and bicuculline 50, 100 and 200 ng/µL in to rostral RNT through guide cannulas at 0800. We did four video-EEG recordings of 8 h, the first one is control, and three additional recordings using an increasing doses scheme every 72 h. Off-line we analyzed the frequency and mean duration of the SWD. We followed the NIH rules for the care and use of experimental animals and the protocol was approved by the University committee. The administration of gaboxadol in the RNT abolished the absence seizures during the first 6 h of EEG recordings with the infusion of 15 and 30 µg ($P<0.001$) with respect to control group. On the other hand, the administration of 100 and 200 ng of bicuculline increased the number of absence seizures for 2 hours ($P<0.05$), with significant reduction on their mean duration ($P<0.001$). The decrease in frequency and duration of absence seizures due to gaboxadol and the corresponding increased induced by bicuculline support the role of rostral RNT in the onset of absence seizures on rat *taiep*, an animal model of the leukodystrophy H-ABC.

Disclosures: J.M. Ibarra-Hernández: None. **C. Cortes:** None. **J.R. Eguibar:** None.

Poster

P140. Networks

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Program #/Poster #: P140.02

Topic: B.10. Epilepsy

Support: NIH Grant NS040337
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Title: The surprising role of the thalamus and corpus callosum during focal to bilateral seizures

Authors: *A. BRODOVSKAYA[1], T. BATABYAL[2], J. KAPUR[3];
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Abstract: Previous studies indicate that the thalamus is essential for seizure maintenance and generalization. Famous models show that focal to bilateral seizures engage commissural connections between two thalamic nuclei to spread contralaterally. However, seizure spread from the seizure focus to the contralateral cortex has not been previously traced. We propose that seizures spread from the ipsilateral thalamus to the contralateral by engaging the corpus callosum. To initiate frontal lobe focal to bilateral seizures, we implanted 1.7 mg of cobalt wire in the right (ipsilateral) premotor cortex. We recorded local field potentials from the bilateral premotor cortex and bilateral ventrolateral thalamic nucleus (VL) simultaneously to determine seizure onset latencies. We injected AAV9 GFP at the seizure focus or in the ipsilateral thalamus in activity reporter TRAP2 mice to label anatomical projections and seizure activated cells at the same time. We also performed callosotomy and used chemogenetics and optogenetics to investigate the role of the corpus callosum and thalamus in the spread of frontal lobe focal to bilateral seizures. Surprisingly, we found that seizures spread faster to the contralateral cortex than to the contralateral thalamus. 53% of all seizures required less than 200 ms to arrive at the contralateral thalamic nucleus VL (59.36 ± 5.61 ms), whereas 47% of seizures required several seconds to arrive there (4.47 ± 0.65 s, 30 seizures, n=11). However, 100% of all seizures required less than 200 ms to arrive at the contralateral cortex (25.49 ± 3.00 ms, 64 seizures, n=16), supporting the role of the corpus callosum in seizure generalization. We injected AAV9 GFP at the seizure focus in TRAP2 mice and found extensive GFP and tdTomato expression in the corpus callosum. We injected AAV9 GFP in the ipsilateral thalamus but did not find anatomical projections to the contralateral thalamus from the ipsilateral, explaining onset delay in the contralateral thalamus. TRAP2 mice also did not express tdTomato in the contralateral thalamus because seizures that arrived there were shorter due to the onset delay. Anterior callosotomy stopped the contralateral spread of initial seizures. However, as a mouse experienced more seizures, plasticity and new network formation allowed faster, genu-independent, seizure onset in the contralateral cortex. We also inhibited ipsilateral VL using chemogenetics and optogenetics. Surprisingly, inhibition of the ipsilateral thalamus did not stop the contralateral spread of seizures. We show that frontal lobe seizures use the corpus callosum for focal to bilateral spread rather than commissural connections between two thalami.

Disclosures: A. Brodovskaya: None. **T. Batabyal:** None. **J. Kapur:** None.

Poster

P140. Networks

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P140.03

Topic: B.10. Epilepsy

Title: Hemodynamics- and electrophysiology-derived connectome dynamics show spatial co-occurrences at frequency-specific, temporally-sparse, and asynchronous times

Authors: *P. MOSTAME[1], J. WIRSICH[2], T. ALDERSON[3], B. RIDLEY[4], S. VULLIEMOZ[2], M. GUYE[4], L. LEMIEUX[5], S. SADAGHIANI[6];
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Abstract: To maintain the malleable nature of cognition, functional connectivity (FC) spatial organization recurrently reconfigures over time between distinct spatial patterns called "Connectome states". Dynamic spatial reconfigurations of hemodynamics- (fMRI) and electrophysiology-derived (MEG/EEG/iEEG) FC are - independently- shown to be informative to understand the brain. Because of a focus on methodological cross-validation of each data modality, prior multi-modal work has been largely biased towards providing evidence for an association between hemodynamics- and electrophysiology-derived FC over time and/or space. However, beyond a certain level of robust convergence, there is also a considerable divergence between hemodynamics- and electrophysiology-derived FC. Here, we asked to what degree fMRI and electrophysiology capture overlapping connectivity processes by comparing their connectome dynamics. We used rare concurrent fMRI-intracranial EEG (iEEG) resting state recordings of 9 subjects undergoing presurgical evaluations for epilepsy treatment [1]. Importantly, we generalized our findings to whole-brain connectomes of healthy subjects using a secondary concurrent fMRI and source-localized EEG data [2]. Amplitude coupling (AmpC) and phase coupling (PhC) (collectively, iEEG-derived FC) were measured for each canonical frequency band at every 100ms. fMRI-derived FC was calculated using spontaneous multiplication of z-scored ROI signals [3], and up-sampled to the temporal resolution of iEEG-derived FC. Over all frequencies, fMRI-derived FC was spatially similar to iEEG-derived FC in only ~20% of times, with temporally-sparse frequency-specific occurrences. Importantly, such cross-modal co-occurrences of the connectome states were also observed with the same prevalence when looking at all possible pairs of
asynchronous times in fMRI and iEEG. This observation indicates that fMRI and iEEG connectome dynamics are driven by distinct neurobiological connectivity processes that exhibits similar connectome states at different times (i.e. asynchronously linked). However using clustering analysis, we found that the spatial co-occurrences of fMRI and iEEG FC arise at sparse times at which the brain connectome reconfigure into a specific set of discrete connectome states. Altogether, we found that fMRI and (i)EEG FC show spatial co-occurrences at frequency-specific, temporally-sparse, and asynchronous times. We conclude that hemodynamic- and electrophysiology-derived connectivity may be envisaged as capturing distinct aspects of brain function, rather than intermodal measurements of the same phenomenon.

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Poster

P140. Networks

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Program #/Poster #: P140.04

Topic: B.10. Epilepsy

Support: Wellcome Trust Grant: 204014/Z/16/Z

Title: Higher order thalamic nuclei factilitate the cortical generalisation of Spike and Wave Discharges

Authors: *Z. ATHERTON[1], P. SERE[2], G. KOZÁK[2], T. FÖLDI[2], A. BERÉNYI[2], V. CRUNELLI[1], M. LÖRINCZ[2];
[1]Cardiff Univ., Cardiff, United Kingdom; [2]Univ. of Szeged, Szeged, Hungary

Abstract: Spike and wave discharges (SWD), generated by the cortico-thalamo-cortical (CTC) network, are pathological oscillations and the hallmark of absence seizures. SWD begin in cortical initiation networks in both humans and animal models, including the Genetic Absence Epilepsy Rats from Strasbourg (GAERS), where they initiate in the primary Somatosensory cortex (S1). The mechanisms of how SWD rapidly spread across the brain has not been explored in detail and it remains unknown how trans-thalamic corico-cortical communication contributes to this process. We have now investigated the role of higher order thalamic (HO) nulcei in the generalisation of SWD in freely moving GAERS. The diffuse connectivity of HO nuclei, their known anatomical interactions with S1 and their altered synaptic anatomy in GAERS make these nuclei potential candidates for the rapid cortical spreading of SWD. Local field potential recordings of different HO nuclei and different cortical regions revealed a novel feature of SWD: the synchrony in cortical regions located far from S1 (such as primary Visual cortex, V1) transiently disappeared, i.e. there are short breaks in SWD (names SWD-breaks). Additionally, relatively brief SWD would occur only in S1 or in S1 and the neighbouring primary Motor cortex (M1), but not elsewhere. These spontaneous events provded a unique mechanistic insight since they represent unsuccessful maintenance and generalisation of SWD.
Local inhibition of different HO nuclei with muscimol increased the delay of SWD propogation and the occurance and duration of SWD-breaks. Moreover, HO nuclei single unit recordings revealed heterogeneity of burst firing dynamics during SWD of putative excitatory neurons. Noteably, all neurons exhibited a switch from tonic to burst firing before the generalisation of SWD, but responded differently during ictal periods, V1 SWD-breaks and non-generalised SWD events. These results support the view that trans-thalamic cortical communication is utilised in the initial propogation of SWD and has an active role in maintaining cortical sychrony throughout the paroxsysmal activity.

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Poster

P140. Networks

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Program #/Poster #: P140.05

Topic: B.10. Epilepsy

Title: Classifying interictal epileptiform spikes and sharp-wave ripples in SEEG recordings in the hippocampus of patients with temporal lobe epilepsy

Authors: *N. MORTAZAVI, M. KHAKI, G. GILMORE, J. BURNEO, D. STEVEN, J. MARTINEZ-TRUJILLO, A. SULER-MARTI;
Univ. of Western Ontario, London, ON, Canada

Abstract: *Nasim Mortazavi, Milad Khaki, Greydon Gilmore, Jorge G Burneo, David A Steven, Julio Martinez-Trujillo, Ana Suller Marti.* **Purpose:**Interictal epileptiform spikes (IISs) and Sharp Wave ripples (SWRs) are pathological and physiological events in patients with epilepsy. Although these two events may display similar temporal frequency patterns, they carry different energy in different frequency bands. We study a group of epilepsy patients implanted with SEEG depth electrodes to identify interictal abnormalities IISs and differentiate them from SWRs using a new algorithm. Our algorithm uses these frequency-specific IISs and SWR waveforms to detect and discriminate between them. **Method:**We analyzed the SEEG recordings obtained from patients with medically-resistant epilepsy implanted with depth electrodes at the Western University Hospital in Epilepsy Unit. The data were cleaned, denoised, montaged and finally segmented as sleep intervals and observed Ictals. The signal waveform and its power were extracted symmetrically in non-overlapping intervals of 500 ms for event detection. Each waveform's power across all detected spikes was computed and clustered based on their energy distributions. **Results:**We examined the efficiency of an automatic SWRs detection algorithm using 320 hours of data from temporal lobe epilepsy patients implanted with depth electrodes. Our finding shows that the rate of the putative IISs and SWRs were anticorrelated per day. Also, IISs class was mostly expected to happen between seizures. However, the putative SWR class in the hippocampus was most likely to occur in the awake state. Furthermore, the correlation between the frequency bands indicates that putative SWRs and putative IISs have the most overlap around [65-70] Hz centre frequency. Moreover, the temporal extent of IISs and SWR waveform distributions indicate that IEDs are less temporally localized (temporal heterogeneity). However, SWRs appeared localized and, as a result, as one single distribution**Conclusion:**Our algorithm detected and successfully distinguished IISs from SWRs based on their spectro-temporal characteristics. Our results indicate that the two neural events are prone to appear at

different time intervals. Moreover, the clustering shows that the statistical approach can define these events using their temporal extension (peak width), frequency, and energy patterns. This ratio is subject to change in different sessions and is affected by the number of seizures per day. The finding elaborates that putative SWRs are less heterogeneous compared to IISs.

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Poster

P140. Networks

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Program #/Poster #: P140.06

Topic: B.10. Epilepsy

Support: NIH NS094399-06

Title: Functional network analysis of high frequency oscillations to identify location of seizure onset zone

Authors: *J. LIN;
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Abstract: Since their discovery, pathological high frequency oscillations (HFO) has been implicated to be a promising biomarker for seizure focuses known as the seizure onset zone (SOZ). A number of studies have shown the rate at which these small amplitude, fast (80-500 Hz) waveforms occur in epileptic patients tend to correlate with epileptic tissue. However, HFO research has been primarily focused on examining features from single channels, such as identifying which have the highest HFO rates. On the other hand, epilepsy has always been described as a network disorder, which would require the understanding of the relationships between the HFOs detected on different channels in order for clinicians to make substantial clinical decisions. Therefore, we strive to expand the understanding of the relationship between HFOs and epilepsy through an identification and characterization of functional HFO networks in epileptic patients. We hypothesize that the characterization of HFO networks will yield complementary information in identifying the epileptic seizure onset zone. To do so, we first evaluate the pairwise relationships between channels during HFO detections in order to generate a set of functional connectivity networks, focusing on the relative lead/lag patterns of co-occurring HFO events. Then, we characterize the functional role of each node within the network with a suite of centrality and ranking algorithms, allowing us to pinpoint the nodes that may play a causal role in generating HFO activity. Our results show that nodes with the highest centrality tend to correlate with clinically defined seizure onset zones. Finally, we compared our analysis with the surgical outcomes of patients and found that it was able to identify patients that went on

to have unsuccessful surgeries, based solely on interictal HFO data that were not available to the treating clinicians. These results provide a new perspective on evaluating the role of HFOs in epilepsy which could prove to be useful in future clinical planning.

Disclosures: J. Lin: None.

Poster

P140. Networks

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P140.07

Topic: B.10. Epilepsy

Title: Interictal activity modulates large-scale cortical networks in children with epilepsy

Authors: *W. KIM[1], **H. YU**[2], D. K. PARK[3], F. A. PROVENZANO[1], D. KHODAGHOLY[2], J. N. GELINAS[1];
[1]Dept. of Neurol., [2]Dept. of Electrical Engin., [3]Dept. of Biomed. engineering, Columbia Univ., New York, NY

Abstract: OBJECTIVE: Intercortical communication is disrupted in patients with focal epilepsy, affecting the functions of anatomically remote brain areas. In the adult brain, pathologic oscillatory coupling between interictal epileptiform discharges (IEDs) and sleep spindles occurs across large-scale networks. Here, we investigate such oscillatory coupling in the developing brain. METHODS: Invasive subdural electroencephalogram (iEEG), presurgical magnetic resonance imaging (MRI) and postsurgical computed tomography (CT) of pediatric patients who underwent investigation due to refractory focal epilepsy were collected and analyzed. IEDs, high frequency oscillations (HFOs) and spindles were detected and cross-correlated to elucidate spatiotemporal relationships. Electrode localization was obtained through co-registration of imaging studies to a standard template. RESULTS: Total of twenty-nine patients (13 males and 16 females) were recruited for analysis. The mean age of iEEG study was 12.7 years old (range 3-21). Mean cortical area of coverage was 58cm2 (range 40-114). 52% (n=15) were temporal lobe exploration and 48% (n=14) were extratemporal lobe exploration. Prominent HFOs were observed in 48% (n=14). IEDs and HFOs demonstrated coupling across local and large-scale networks, with patient specific patterns. In addition, IEDs and HFOs could be phase-locked to physiologic-appearing spindles in distant brain regions. CONCLUSIONS: Pathologic oscillatory coupling is prevalent in immature epileptic networks, but with a diversity of patterns. Our findings suggest that IED-spindle coupling may shape development of interictal global network dysfunction in pediatric epilepsy patients.

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Poster

P172. Glia-Neuron Interactions in Injury and Disease: Synapses

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P172.01

Topic: B.11. Glial Mechanisms

Support: R01 A124677
R25 NS105141
NIH-NINDS F99NS120596

Title: Complement-dependent neuron-glia interactions mediate the loss of perisomatic inhibitory synapses in Toxoplasma gondii infection

Authors: *G. CARRILLO[1], V. BALLARD[1], T. GLAUSEN[2], Z. BOONE[1], J. TEAMER[1], C. HINKSON[3], E. WOHLFERT[2], I. J. BLADER[2], M. A. FOX[1,3];
[1]Fralin Biomed. Res. Inst., Roanoke, VA; [2]Univ. at Buffalo, Buffalo, NY; [3]Virginia Tech. Carilion Sch. of Med., Roanoke, VA

Abstract: Prolonged infection and inflammation within the brain can alter the connectivity and function of neuronal circuits. The intracellular parasite, *Toxoplasma gondii*, is one pathogen that can chronically infect the brain and lead to encephalitis and seizures. Currently, 1 in 3 humans are infected with *Toxoplasma gondii* worldwide, and these infections have been identified as a considerable risk factor for developing complex neurological and psychiatric disorders that arise from alterations in assembly and maintenance of synapses, such as schizophrenia. To directly assess changes in inhibitory synapses, we employed Serial Block Face Scanning Electron Microcopy and quantified perisomatic synapses in neocortex and hippocampus following parasitic infection. Ultrastructural analyses, in combination with genetic and immunohistochemical tools, revealed that persistent infection not only led to a significant loss of inhibitory perisomatic synapses, it also induced the ensheathment of neuronal somata and perisomatic nerve terminals by activated myeloid-derived cells (including microglia), suggesting they may displace or phagocytose synaptic elements in long-term infection. How might microglia contribute to inhibitory synapse loss in parasitic infection? Based on the well-established role of the innate complement cascade contributing to synaptic refinement in the developing brain and synapse loss in the diseased brain, we assessed complement components in parasitic infection. Indeed, C1q and C3 are highly upregulated in neocortex and hippocampus following persistent *Toxoplasma gondii* infection. Therefore, we hypothesized that these components may be required for microglia-induced synapse loss during long-term infection. Using transgenic and knockout mouse models, our current studies demonstrate that C3 is, in part, required for phagocytic microglia to ensheath neurons in long-term infection, likely leading to the loss of inhibitory perisomatic synapses. Together, these studies highlight novel ways in which immune

molecules regulate neuron-glia interactions to alter mature inhibitory circuits in parasitic brain infection.

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Poster

P172. Glia-Neuron Interactions in Injury and Disease: Synapses

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P172.02

Topic: B.11. Glial Mechanisms

Support: 1R01NS105742

Title: Astrocyte-secreted chordin-like 1 regulates spine density after ischemic stroke in mice

Authors: *E. BLANCO-SUAREZ[1,2], N. J. ALLEN[2];
[1]Thomas Jefferson Univ., Philadelphia, PA; [2]Salk Inst. for Biol. Studies, La Jolla, CA

Abstract: Focal ischemic stroke is a condition characterized by the interruption of the blood flow to a specific region of the brain. Ischemic stroke causes alterations in the peri-infarct area, the surroundings of the core of the injury, including acute loss and morphological changes of dendritic spines. This drives modifications in the synaptic plasticity that can determine the degree of recovery from ischemic stroke. Astrocytes are known to play detrimental and neuroprotective roles at different stages post-stroke, yet we lack a clear understanding of the mechanisms by which they exert those functions. Recently, we identified Chordin-like 1 (Chrdl1) as the astrocyte-secreted factor that promotes synaptic maturation in the mouse visual cortex, limiting plasticity. Chrdl1 KO mice exhibit enhanced experience-dependent plasticity in a paradigm of visual deprivation, and it has been previously shown that blocking plasticity-limiting molecules lead to better recovery from ischemic stroke. Using a photothrombotic *in vivo* model of focal ischemic stroke in the visual cortex, I analyzed the effects of Chrdl1 absence on structural plasticity (spine density and morphology), astrocyte and microglia reactivity, and injury volume progression. The results from this study suggest that Chrdl1 plays a role in regulating synaptic plasticity in response to acute injury, such as focal brain ischemia. Astrocyte-secreted factors like Chrdl1 represent a new target to prevent detrimental effects of acute injury and to enhance endogenous plasticity to compensate at later stages for the damage caused at early phases after focal ischemic stroke.

Disclosures: E. Blanco-Suarez: None. **N.J. Allen:** None.

Poster

P172. Glia-Neuron Interactions in Injury and Disease: Synapses

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Topic: B.11. Glial Mechanisms

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Medical Technology Enterprise Consortium

Title: Interrogating microglial engulfment of synaptic terminals using FEAST

Authors: *L. DISSING-OLESEN, A. J. WALKER, Q. FANG, D. K. WILTON, I. DAS, L. BENOWITZ, B. STEVENS;
Boston Children's Hosp., Boston, MA

Abstract: Engulfment of synaptic material is a key microglia function in health and disease, however, thoroughly validated tools that facilitate rapid, high-throughput, and unbiased quantification of this process in different paradigms are lacking. We therefore developed FEAST *(Flow cytometric Engulfment Assay for Synaptic Terminals)* to facilitate rapid analysis of *in vivo* engulfment of synaptic material by thousands of microglia at single cell resolution. We optimized FEAST for two different analyses of *in vivo* engulfment: quantification of fluorescent neuronal material inside live microglia and of engulfed endogenous synaptic proteins within fixed microglia. We conducted multiple control experiments to verify that the majority of neuronal material quantified within each microglial cell was engulfed *in vivo* and that *ex vivo* engulfment and technical artifacts have been substantially reduced. We confirmed the specificity of FEAST by interrogating engulfment of eye-specific inputs after optic nerve crush. Additionally, we applied FEAST to discover that depletion of the microbiome leads to increased engulfment by microglia, underscoring the applicability of this technique to novel questions in the field of neuro-immunology. Conceptually, FEAST is not restricted to microglial engulfment of synaptic terminals in the central nervous system and can be expanded to allow quantification of engulfment of any protein by any cell type across different tissues and model organisms. FEAST can also be combined with emerging -omics analyses, providing a functional readout that complements our understanding of the phenotypic heterogeneity of myeloid cells across different paradigms. Ultimately, FEAST has the potential to provide critical insights into neuro-immune interactions that shape development, homeostasis, and disease.

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Poster

P172. Glia-Neuron Interactions in Injury and Disease: Synapses

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Topic: B.11. Glial Mechanisms

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Title: Glial engulfment of synapses is enhanced in a mouse model of Fragile X Syndrome

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Abstract: Fragile X Syndrome (FXS) is the most common single-gene cause of both autism and intellectual disability and is caused by the transcriptional silencing of *Fmr1*, resulting in loss of FMRP. While neuronal mechanisms underlying aspects of FXS have been identified, therapeutic strategies targeting these mechanisms have been largely unsuccessful, suggesting that our current focus is missing an essential piece of the puzzle. One such missing piece may be glia, or the non-neuronal cells of the brain, which express FMRP and which our lab and others have recently identified as involved in FXS-associated phenotypes. We have leveraged the unique advantages of the developing visual (retinogeniculate) system, a classic model for studying synapse growth and refinement, to test the hypothesis that loss of FMRP alters glia-dependent mechanisms of synapse refinement. Retinal ganglion cells (RGCs) extend from the retina and synapse onto the dorsal lateral geniculate nucleus (dLGN) of the brain, initially forming an excess of synaptic connections that are subsequently eliminated in a stereotyped manner to segregate inputs from each eye. This refinement process requires astrocytic and microglial engulfment of synapses. We found that RGC segregation is enhanced in the *Fmr1* KO dLGN at P7, a time of peak refinement. Additionally, excitatory RGC synapses in the dLGN appear reduced in size at this age. To identify the molecular mechanisms underlying this phenotype, we performed differential single-nucleus RNA sequencing (snSeq) of the P7 *Fmr1* KO and WT dLGN and identified

differentially expressed genes in KO vs WT within different dLGN cell types. Unsupervised analysis of these data indicated that *Fmr1* KO microglia and astrocytes show an upregulation of genes associated with glial phagocytosis. Finally, we found that both microglial and astrocytic engulfment of RGC synapses is upregulated *in vivo* in the developing *Fmr1* KO dLGN. Altogether, these data indicate that loss of FMRP results in aberrantly enhanced glial engulfment of synapses during neural circuit development, demonstrating a novel potential therapeutic target for the treatment of FXS.

Disclosures: M. Lee: None. **L. Cheadle:** None. **A. Vo:** None. **A. Yadav:** None. **M. Weisenhaus:** None. **V. Menon:** None. **M.E. Greenberg:** None. **M.M. Shirasu-Hiza:** None. **C. Mason:** None.

Poster

P172. Glia-Neuron Interactions in Injury and Disease: Synapses

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P172.05

Topic: B.11. Glial Mechanisms

Support: NIH R01-NS-079166
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NIDA/NIH R01-DA-036165

Title: Glia-mediated synapse pruning in HIV gp120 transgenic mice

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Abstract: Synaptic loss is implicated in the pathogenesis of HIV-associated neurological disorders (HAND) such as pain and dementia. We previously reported that synaptic degeneration in the spinal dorsal horn is associated with the development of chronic pain in HIV patients. This pathology is also recapitulated in mouse models of HIV-associated pain generated by the HIV envelope glycoprotein, gp120. Glial activation is a common feature in HIV patients and animal models of HIV-associated pain. A recent study from our lab detailed a crucial signaling pathway required for gp120 to induce microglial synapse degeneration. However, the underlying synapse elimination mechanism remains elusive. Recent studies and our preliminary data have revealed a critical role for glia in synapse pruning, therefore we hypothesize that reactive microglia and astrocytes play crucial roles in HIV-associated synapse loss. In this study, we aim to investigate

synapse pruning as a mechanism by which glial cells contribute directly to synapse loss in an HIV animal model. To do so, we compared glial synapse engulfment between wild type and gp120 transgenic mice (gp120tg) in the cortex and spinal dorsal horn. The gp120tg mice express HIV LAV gp120 under the GFAP promoter resulting in expression and secretion of gp120 from astrocytes, a reservoir for HIV in the CNS. GP120tg and age-matched WT mice were euthanized at 5 months of age (n=3 per group). Sex differences were not assessed. Brain and spine tissue were collected for immunofluorescent staining. Tissue sections were stained for pre-synaptic Vglut1, post-synaptic Shank1, lysosome marker LAMP1, and microglia marker iba-1 or astrocyte marker GFAP. Three sections were analyzed per animal and all glial cells within each section analyzed. 3D reconstruction of confocal z-stack images showed increased glial volume and cell body count suggesting activation of microglia and astrocytes in both tissues studied. In the cortex, astrocyte engulfment of both Shank1 and vGlut1 increased from WT to gp120tg ($p=.002$, $p=1.8e-7$), and microglia engulfment of the same markers also appeared to increase from WT to gp120tg ($p=.09$, $p=.08$). In the spinal dorsal horn, compared to WT, gp120tg, astrocyte engulfment of Shank1 appeared to increase ($p=.05$), vGlut1 engulfment increased ($p=.0049$), and microglia engulfment of Shank1 increased ($p=.008$). However, engulfment of vGlut1 by microglia did not appear to increase. A two sample t-test was performed for statistical analysis between groups. These results suggest that synaptic pruning by glial cells is a vital contributor to synapse loss in HAND.

Disclosures: Z. Watson: None. **J. Zheng:** None. **M. Spurgat:** None. **W. Ru:** None. **S. Tang:** None.

Poster

P172. Glia-Neuron Interactions in Injury and Disease: Synapses

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P172.06

Topic: B.11. Glial Mechanisms

Title: An astrocytic role in regulating dendritic spine maturation in UBE3A-dependent autism spectrum disorder

Authors: *Z. GARDNER, O. HOLBROOK, H.-Y. MAN;
Boston Univ., Boston, MA

Abstract: An astrocytic role in regulating dendritic spine maturation in *UBE3A*-dependent autism spectrum disorder
Zachary Gardner, Otto Holbrook, Heng-Ye Man
Autism spectrum disorders (ASDs) are a diverse class of neurodevelopmental disorders with various aberrant cellular phenotypes such as dysfunctional neurotransmission and irregular neuronal morphology. ASDs have a broad underlying genetic background with many genes

linked to their etiology. *UBE3A* has been identified as a top gene candidate to ASD, and overexpression of *UBE3A* via copy-number variation confers hallmark ASD behaviors in humans and transgenic mice. Our previous work revealed that in the *Ube3A*-ASD mouse model (*Ube3A* 2X Tg, or simply "2X Tg"), synapse formation was negatively affected. However, the cellular and molecular mechanisms underlying the synaptic dysregulation remain unknown. We found that selective overexpression of *Ube3A* in neurons failed to induce changes in synapse development. In contrast, overexpression of *Ube3A* in glia resulted in alterations in spines and synapses. Further, we identified thrombospondin-2 (TSP2), a secreted glial molecule involved in synaptogenesis, which we implicate in the defective synapse formation. *Ube3A* overexpression confers a loss of transcriptional down-regulation of TSP2 in astrocytes, and the medium of glia cultures with *Ube3A* overexpression was sufficient to trigger synaptic changes similar to that observed in mixed cultures from 2X Tg brains. Importantly, supplement of TSP2 to the 2X Tg cultures was able to rescue the synaptic defects. These findings indicate that an increase in *Ube3A* dosage in astrocytes contributes to the synaptic abnormalities in neurons.

Disclosures: Z. Gardner: None. **O. Holbrook:** None. **H. Man:** None.

Poster

P172. Glia-Neuron Interactions in Injury and Disease: Synapses

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Program #/Poster #: P172.07

Topic: B.11. Glial Mechanisms

Support: National Institutes of Health (2U54GM104942-03) to MLR
Department of Veteran Affairs Career Development Award (BX002505) to MLR

Title: Adolescent binge ethanol exposure induces changes in astrocyte morphology and synaptic colocalization disrupting neuronal-astrocyte communication that persists into early adulthood

Authors: *C. D. WALKER[1], H. G. SEXTON[2], B. J. HENDERSON[3], M.-L. RISHER[4]; [1]Biomed. Res., [2]Joan C. Edwards Sch. of Medicine, Marshall Univ., Huntington, WV; [3]Biomed. Sci., Joan C Edwards Sch. of Med. at Marshall Univ., Huntington, WV; [4]Biomed. Sci., Joan C. Edwards Sch. of Med., Huntington, WV

Abstract: Adolescence, a critical time of neuronal development, is characterized as a transitional period between childhood and adulthood that is associated with increased freedom, self-exploration, and participation in novel experiences. Alcohol consumption in the form of binge drinking is highly prevalent among adolescents and emerging adults, increasing the risk of alcohol dependence and cognitive impairment later in life. Animal models have shown disruption of neuronal structure and function as a result of repeated binge-level ethanol (EtOH) exposure. However, the underlying mechanisms that elicit these changes are not well understood.

Furthermore, the contribution of astrocytes on EtOH induced neuronal dysfunction is unknown. Astrocytes have complex morphologies with extensive perisynaptic astrocyte processes (PAPs) that ensheathe pre- and post-synaptic terminals and play an essential role in synaptic maintenance and signal transmission. Here we investigate the consequences of adolescent binge EtOH exposure on astrocyte morphology, PAP-synaptic proximity, and neuronal-to-astrocyte communication in the form of astrocyte Ca^{2+} activity. We hypothesize that adolescent binge EtOH exposure induces changes in astrocyte morphology that results in a loss of PAP-synaptic proximity subsequently impairing astrocyte responsivity to neuronal activation.
Male Sprague Dawley rats received hippocampal intracranial injections of an astrocyte-specific adeno-associated GFP virus or an astrocyte-specific Ca^{2+} indicator GCaMP6f. Beginning at PND30, animals received intermittent EtOH (5g/kg) or water gavage over 16 days followed by a 26-day washout period. On PND70 acute hippocampal slices were prepared for immunohistochemistry (IHC, astrocyte morphology) or electrophysiology (neuronal-astrocyte responsivity). IHC was performed on GFP+ slices using the post synaptic density marker PSD95. GFP+ astrocytes in the CA1 hippocampus were imaged using confocal microscopy and then reconstructed using IMARIS-Bitplane. Astrocyte Ca^{2+} activity was recorded from GCaMP6F+ CA1 hippocampal astrocytes following Schaffer Collateral stimulation.
Our data demonstrate that adolescent intermittent binge EtOH results in a protracted decrease in astrocyte volume and PAP-synaptic colocalization that correlates with decreased astrocyte responsivity to neuronal activation as demonstrated by a reduction in astrocyte Ca^{2+} activity. These findings contribute to our understanding of how adolescent EtOH disrupts neuronal-astrocyte communication, driving astrocytic and synaptic dysfunction that persist into adulthood.

Disclosures: C.D. Walker: None. **H.G. Sexton:** None. **B.J. Henderson:** None. **M. Risher:** None.

Poster

P176. Human and Animal Studies and Therapeutics: Demylinating Disorders

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P176.01

Topic: B.12. Demyelinating Disorders

Support: NIH Grant R01NS107523

Title: Interleukin 4-induced 1 (IL4i1) modulates the metabolic microenvironment in demyelinated lesions to facilitate remyelination

Authors: *J. HU, H. C. OFT, V. N. SMITH, M. KIM, L. ROSKO, G. S. MELCHOR, M. BAYDYUK, J. K. HUANG;
Georgetown Univ., Washington, DC

Abstract: Multiple sclerosis (MS) is an immune-mediated disease in the central nervous system (CNS) that is characterized by demyelination, axonal injury, and neuronal degeneration. Our previous study found that interleukin 4-induced 1 (IL4i1), an L-amino acid oxidase expressed by microglia/macrophages, is upregulated at the onset of recovery in animal models of MS, promoting inflammation resolution and remyelination. However, it remains unclear what are the downstream targets of IL4i1 and how IL4i1 facilitates the remyelination process. Here, we show that recombinant IL4i1 treatment inhibits the amino acid-dependent mammalian target of rapamycin complex 1 (mTORC1) pathway in immune cells. Deletion of IL4i1 in mice resulted in increased mTORC1 activity in myeloid cells as well as prolonged inflammation in lysolecithin-induced focal demyelination and experimental autoimmune encephalomyelitis (EAE) animal models. Moreover, systemic administration of L-phenylalanine, a major IL4i1 substrate, prolonged inflammation and inhibited remyelination in mice following focal demyelination. Through mass spectrometry analysis of dissected demyelinated tissues, we observed that lesions from IL4i1$^{-/-}$ mice exhibited decreased levels of α-keto acids, corresponding to the failure of amino acid metabolism in the absence of IL4i1. We also found that IL4i1-derived α-keto acids modulate inflammation by activating the aryl hydrocarbon receptor (AHR) pathway in vitro. Moreover, IL4i1$^{-/-}$ mice displayed a reduction of AHR activity in myeloid cells. Our results suggest that IL4i1 modulates inflammation in the demyelinated lesion microenvironment through inhibiting mTOR signaling by oxidizing amino acids, while generating α-keto acids that activate AHR signaling in myeloid cells to facilitate remyelination.

Disclosures: J. Hu: None. **H.C. Oft:** None. **V.N. Smith:** None. **M. Kim:** None. **L. Rosko:** None. **G.S. Melchor:** None. **M. Baydyuk:** None. **J.K. Huang:** None.

Poster

P176. Human and Animal Studies and Therapeutics: Demylinating Disorders

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P176.02

Topic: B.12. Demyelinating Disorders

Title: Thyroid receptor beta-selective agonist enhances functional brain delivery and accelerates remyelination in in vitro and in vivo rodent demyelination models.

Authors: M. B. WOERNER[1], J. VIVIAN[1], A. ALAVI[1], M. SHANKARAN[2], E. NYANGAU[2], M. MATTHEWS[2], M. HELLERSTEIN[2], *R. GANDHI[1], C. BEALS[1], B. A. STEARNS[1], D. A. MACKENNA[1];
[1]Autobahn Therapeut., San Diego, CA; [2]Univ. of California at Berkeley, Berkeley, CA

Abstract: Demyelinating diseases are characterized by loss of myelin in the CNS. There are no effective treatments to regenerate damaged myelin, and natural remyelination is often inhibited or impaired. Thyroid hormone (T3) is known to induce oligodendrocyte differentiation and

myelinogenesis *in vivo*. Brain-directed thyromimetics have potential to enhance remyelination in the treatment of demyelinating diseases such as multiple sclerosis.
LL-341070 is a prodrug that delivers a highly potent and selective small molecule agonist for the thyroid hormone receptor beta (TRβ). LL-341070 demonstrates enhanced brain delivery and induction of thyroid-responsive genes *in vivo*. Studies to profile LL-341070 were performed in cellular (OPC differentiation) and rodent demyelination models (cuprizone diet & experimental autoimmune encephalitis (EAE)).
The active metabolite of the LL-341070 prodrug enhanced oligodendrocyte differentiation *in vitro* in an oligodendrocyte progenitor cell assay (EC_{50} = 1.4 nM).
T_3 has been shown to enhance the rate of remyelination in a rat cuprizone model. We assessed the ability of thyromimetics to accelerate the remyelination process *in vivo* by measuring the fractional synthesis of 24-hydroxycholesterol (24-OHC) in the brains of rats following cuprizone-induced demyelination. 24-OHC is a primary metabolite of *de novo* cholesterol synthesis in the CNS, which is required for efficient remyelination, is released into circulation from brain, and may represent a circulating biomarker of brain cholesterol synthesis and remyelination.
At withdrawal from cuprizone diet, rats were provided deuterated water with administration of LL-341070 at 30 or 100 µg/kg for 3 weeks, then 24-OHC deuterium enrichment and labeling pattern were measured in cortex and corpus callosum. LL-341070 induced a dose-dependent increase in deuterated 24-OHC compared to vehicle controls, suggesting an increased rate of myelin synthesis. Additionally, expression of thyroid-responsive genes increased in the brains of treated animals, indicating active engagement with TRβ.
In an EAE mouse model, LL-341070 administered daily from 3 to 300 µg/kg in a prophylactic paradigm dose-dependently improved median day of disease onset and decreased maximum disease severity. Histological analysis of spinal cord 28 days after immunization demonstrated a reduction of inflammatory foci, apoptotic cell count, and reduced area of demyelination by H&E and MBP staining.
In summary, thyromimetic prodrugs demonstrate utility in models of remyelination through enhanced brain delivery *in vivo* and, as such, have potential as valuable therapeutics for demyelination disorders.

Disclosures: M.B. Woerner: A. Employment/Salary (full or part-time):; Autobahn Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Autobahn Therapeutics. **J. Vivian:** A. Employment/Salary (full or part-time):; Autobahn Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Autobahn Therapeutics. **A. Alavi:** A. Employment/Salary (full or part-time):; Autobahn Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Autobahn Therapeutics. **M. Shankaran:** 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.; Autobahn Therapeutics. **E. Nyangau:** 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.; Autobahn Therapeutics. **M. Matthews:** 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.; Autobahn Therapeutics. **M. Hellerstein:** 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.; Autobahn Therapeutics. F. Consulting Fees (e.g., advisory boards); Autobahn Therapeutics. **R. Gandhi:** A. Employment/Salary (full or part-time):; Autobahn Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Autobahn Therapeutics. **C. Beals:** A. Employment/Salary (full or part-time):; Autobahn Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Autobahn Therapeutics. **B.A. Stearns:** A. Employment/Salary (full or part-time):; Autobahn Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Autobahn Therapeutics. **D.A. MacKenna:** A. Employment/Salary (full or part-time):; Autobahn Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Autobahn Therapeutics.

Poster

P176. Human and Animal Studies and Therapeutics: Demylinating Disorders

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P176.03

Topic: B.12. Demyelinating Disorders

Support: NMSS Grant PP-1901-33093
NIH Grant NS097303

Title: Functional changes precede structural changes in the mouse hippocampus following Cuprizone-induced demyelinaition and remyelination

Authors: A. DAS, J. BOROVICKA, D. PATEL, B. D. TRAPP, ***H. DANA**;
Neurosciences, Cleveland Clin. Lerner Res. Inst., Cleveland, OH

Abstract: Damage to the myelin sheath that protects axons in the central nervous system is part of several demyelinating diseases including multiple sclerosis (MS). Many MS patients exhibit repeating episodes of axonal demyelination, which is associated with many of the disease symptoms of motor, visual, and cognitive deficits. In the first stage of the disease, there are also remission periods, where the axons remyelinate and the symptoms are ceased. Current medical treatment focuses on the inflammatory component of MS and unfortunately, is not efficient in

progressive phases of the disease. In this study, we introduce a new method to link the structural demyelination and remyelination processes with functional changes in neuronal activity in the living mouse brain. We used a transgenic mouse line expressing the red genetically encoded calcium indicators jRGECO1a, and induced brain-wide demyelination using the Cuprizone model. We longitudinally recorded the spontaneous activity from thousands of hippocampal neurons using two-photon microscopy for 100 days. In parallel, we monitored longitudinal structural changes in the hippocampal myelin of the same mice using third-harmonic generation. We found that the changes to neuronal activity are relatively rapid and firing rate decreased by ~50% as early as 4 days after the start of the cuprizone diet, whereas the structural changes of the myelin are slower and can be identified only after several weeks. This relationship remained similar upon the termination of cuprizone diet. we identified a partial recovery of the hippocampal activity within several days and slower remyelination process after more than a week. Our study demonstrates an *in vivo* method to detect longitudinal changes during cycles of demyelination and remyelination that can be applied to study the efficacy of new therapeutic treatments to protect the neuronal circuitry.

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Poster

P176. Human and Animal Studies and Therapeutics: Demylinating Disorders

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P176.04

Topic: B.12. Demyelinating Disorders

Title: Myeltracer - a semi-automated software for myeling-ratio quantification

Authors: *T. KAISER[1], H. M. ALLEN[1], O. KWON[1], B. BARAK[2], J. WANG[3], Z. HE[3], M. JIANG[1], G. FENG[1];
[1]MIT, Cambridge, MA; [2]The Sagol Sch. of Neurosci. and The Sch. of Psychological Sci., Tel Aviv University, Israel; [3]Boston Children's Hosp., Boston, MA

Abstract: In the central and peripheral nervous systems, the myelin sheath promotes neuronal signal transduction. The thickness of the myelin sheath changes during development and in disease conditions like multiple sclerosis. Such changes are routinely detected using electron microscopy through *g*-ratio quantification. While *g*-ratio is one of the most critical measurements in myelin studies, a major drawback is that *g*-ratio quantification is extremely laborious and time-consuming. Here, we report the development and validation of MyelTracer, an installable, stand-alone software for semi-automated *g*-ratio quantification based on the Open Computer Vision Library (OpenCV). Compared to manual *g*-ratio quantification, using MyelTracer produces consistent results across multiple tissues and animal ages, as well as in remyelination

after optic nerve crush, and reduces total quantification time by 40-60%. With *g*-ratio measurements via MyelTracer, a known hypomyelination phenotype can be detected in a Williams Syndrome mouse model. MyelTracer is easy to use and freely available for Windows and Mac OS X (https://github.com/HarrisonAllen/MyelTracer).

Disclosures: T. Kaiser: None. **H.M. Allen:** None. **O. Kwon:** None. **B. Barak:** None. **J. Wang:** None. **Z. He:** None. **M. Jiang:** None. **G. Feng:** None.

Poster

P176. Human and Animal Studies and Therapeutics: Demylinating Disorders

Location: Virtual Only

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Program #/Poster #: P176.05

Topic: B.12. Demyelinating Disorders

Support: Center Grant 1I50RX003002-01 from the United States (U.S.) Department of Veterans Affairs Rehabilitation Research and Development Service

Title: Rescuing Cholesterol Efflux Transporter 1 Expression Mitigates Visual and Motor-sensory Impairment In Experimental Autoimmune Encephalomyelitis

Authors: *O. W. GRAMLICH, J. J. ANDERS, B. W. ELWOOD, R. H. KARDON;
Dept. of Ophthalmology & Visual Sci., Univ. of Iowa, Iowa City, IA

Abstract: Background: Acute optic neuritis is the initial symptom in 25-45% of newly diagnosed cases of Multiple Sclerosis (MS). The purpose of our study is to determine whether augmentation of cholesterol processing by systemic administration of gentisic acid (GA) rescues the ocular phenotype in an experimental autoimmune encephalomyelitis (EAE) model. Methods: EAE-related optic neuritis was induced in 52 female C57BL6 mice by immunization with MOG33-55, complete Freund's Adjuvant and pertussis toxin. 22 EAE mice received intraperitoneal administration of gentisic acid weekly (EAE+GA) while another 25 naïve mice served as controls. Clinical progression was monitored according to a 5-point EAE scoring scheme. At day 60, visual acuity, pattern electroretinography (PERG) and retinal ganglion cell (RGC) complex thickness were measured. Grades of demyelination (0-3 grading), cell infiltration (0-4 grading) and expression of Cholesterol efflux transporter 1, ABCA1, in optic nerves and spinal cord were determined. Differences were evaluated using Tukey's post-hoc test. Results: EAE+GA animals show significantly lower motor-sensory impairment (area under the curve EAE:122±4 vs. EAE+GA: 78±5; $p<0.0001$). Treatment also preserved visual acuity (EAE:0.23±0.08c/d vs. EAE+GA: 0.34±0.09c/d; $p<0.0001$), improved the PERG amplitude (EAE:16±4 vs. EAE+GA:20±4 µV; $p=0.03$) and slightly diminished RGC complex thinning (EAE:59±4µm vs. EAE+GA:62±3µm; $p=0.2$) when compared to untreated EAE mice. Demyelination in the optic nerve was less severe in GA treated EAE mice (EAE:2.1±0.8 vs.

EAE+GA: 1.1±0.6; p=0.0001) and fewer infiltrating cells were observed (EAE:2.2±1 vs. EAE+GA: 1±0.5; p<0.0001). ABCA1 was significantly less downregulated in spinal cord tissue in GA treated EAE mice. (fold change EAE: -3.9±1.1 vs. EAE+GA: -2.3±1.2; p=0.003). Clinical progression in treated and untreated EAE animals strongly correlate with decline in PERG amplitude (r=0.81), RGC complex thinning (r=0.9) and optic nerve histopathology (r=0.7). Conclusion: Administration of GA, which increases the expression of the Cholesterol efflux transporter, significantly lessens motor-sensory deficits and corelates with rehabilitation of the visual system in an EAE mouse model of MS. The rescue effect may be related to increased clearing of myelin debris and cholesterol recycling by macrophages after optic neuritis. In summary, this study suggests that interventions to improve cholesterol recycling might be a viable approach to promote rehabilitation in MS.

Disclosures: O.W. Gramlich: Other; Center for the Prevention and Treatment of Visual Loss, Iowa City VA Healthcare System. **J.J. Anders:** A. Employment/Salary (full or part-time):; Center for the Prevention and Treatment of Visual Loss, Iowa City VA Healthcare System. **B.W. Elwood:** Other; Center for the Prevention and Treatment of Visual Loss, Iowa City VA Healthcare System. **R.H. Kardon:** Other; Center for the Prevention and Treatment of Visual Loss, Iowa City VA Healthcare System.

Poster

P176. Human and Animal Studies and Therapeutics: Demylinating Disorders

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P176.06

Topic: B.12. Demyelinating Disorders

Title: The Effects of Myelin Oligodendrocyte Glycoprotein Antibody-Associated Demyelinating Disease on Regional White Matter Volume and Cognitive Function in Children

Authors: P. THOMAS, C. WILSON, M. HUTCHINSON, *W. I. MATTSON, E. E. NELSON; Nationwide Children's Hosp., Columbus, OH

Abstract: The myelin oligodendrocyte glycoprotein antibody (MOG-Ab) was recently discovered as a contributing factor to some cases of white matter demyelination. MOG mediated demyelination has a much larger prevalence in children than adults. Clinically, the condition manifests as encephalopathy, optic neuritis, acute disseminated encephalomyelitis (ADEM), and neuromyelitis optica spectrum disorder (NMOSD). In MOG-Ab+ individuals, this endogenous antibody binds directly to the myelin oligodendrocyte glycoprotein within the central nervous system, directly affecting both oligodendrocytes and myelinated neurons. Both white matter lesions and clinical symptoms of MOG-Ab+ are successfully managed with immunotherapy including steroids and intravenous immunoglobulin, yet relapses in this disease occur. The underlying cellular mechanisms are being heavily researched, but differentiation in clinical

presentation and relapse-remitting periods are not well defined.
In the present study we examined effects of MOG-Ab+ status on regional white matter volume and neuropsychological profile in a group of pediatric patients recruited from a Neuroimmune clinic at a children's hospital. Multiple MRI scans were obtained from clinical assessments performed on 12 children with demyelinating disease and a matched group of healthy controls. A post-treatment neuropsychological battery was conducted looking at verbal fluency, IQ, attention, executive functioning, and fine motor skills. White matter volumes from T1-weighted MRI scans were calculated with the cortical parcellation function in Freesurfer (v7.1.0). Comparisons were made both within and between subjects at the whole brain and regional lobar levels.
Preliminary evidence indicates regional reductions of up to 6% volume in white matter of frontal lobes are evident in many MOG-Ab+ patients, regardless of lesion location. This regional reduction in white matter volume also tracks with the onset, reduction, and relapsing symptomology of focal lesions. Changes in other lobes are less evident. Post-treatment neuropsychological measures indicate near normal function in MOG-Ab+ patients on nearly all measures. These findings contribute to a narrow pool of literature surrounding this disease presentation, clinical impairments, and cognitive functioning outcomes in pediatric populations. These findings suggest neurological impairment may occur beyond localized lesions, but reasons for this focal volume reduction and long-term functional impact is unclear. Further research is needed to examine neurocognitive and functional outcomes to inform multidisciplinary management to improve recovery.

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Poster

P176. Human and Animal Studies and Therapeutics: Demylinating Disorders

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Program #/Poster #: P176.07

Topic: B.12. Demyelinating Disorders

Support: NIH Grant R01NS111715-02
NIH Grant F31-Diversity

Title: Studying the role of autophagy in Krabbe disease

Authors: *N. DHIMAL[1,3], N. WEINSTOCK[1,3], E. HURLEY[4], D. SHIN[2], L. WRABETZ[5,3], M. FELTRI[6,3];
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Abstract: Krabbe disease (KD) is an inherited autosomal recessive, debilitating, lysosomal storage disorder (LSD) caused by mutations in galactosylcerebrosidase (*Galc*) gene. *Galc* deficiency affects both the peripheral nervous system (PNS) and the central nervous system (CNS) and leads to demyelination, neurodegeneration, and death within a couple years. Although therapies that delay the disease exist, there is currently no available cure for KD. Schwann cells-specific deletion of *Galc* (*Galc*-SC-cKO) in mice leads to a PNS pathological phenotype of KD, which includes accumulation of undigested substrates accompanied by demyelination, neurodegeneration, and a neuropathy very similar to the one observed in KD patients (Weinstock et al. 2020, Neuron). Using this validated *Galc*-SC-cKO model, this study aims to investigate autophagy in the PNS to understand how it contributes to development and pathogenesis of KD. Preliminary data indicate that macroautophagy (or autophagy) is defective in Schwann Cells (SC) when GALC is absent. Western blot results suggest that autophagy is increased at the beginning of the disease, but it halts and decreases during disease progression. Autophagosome accumulation in SCs is observed under electron microscopy (EM) during disease progression. Furthermore, using a pH-sensitive fluorescent LC3 autophagy reporter mouse system, we see a significant increase in autophagosome accumulation in SC from *Galc*-SC-cKO *in vivo*, suggesting impaired fusion of autophagosomes with lysosomes in those cells. Thus, we plan to manipulate autophagy in *Galc*-SC-cKO mice in vivo, and this work is currently in progress. Understanding specific mechanistic changes in degradative pathways could help pinpoint the cause and timing of substrate accumulation in KD and other LSDs. Such studies may also be relevant to other more common diseases caused by failure in lysosomes, autophagy, and proteostasis.

Disclosures: N. Dhimal: None. **N. Weinstock:** None. **E. Hurley:** None. **D. Shin:** None. **L. Wrabetz:** None. **M. Feltri:** None.

Poster

P187. Brain Injury and Wellness: Pharmacological Interventions

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P187.01

Topic: C.01. Brain Wellness and Aging

Title: The influence of LCGA-17 on behavior and brain elongation factor 2 levels in mice with strong features of dominance and submissiveness

Authors: *A. MALYSHEV[1], E. SHMERKIN[2], A. KLINOV[2], L. VINNIKOVA[2], D. BEGMATOVA[2], D. SUR[2], N. MITKIN[1], I. SUKHANOVA[1], V. GEDZUN[1], V. PAVSHINTSEV[1], I. DORONIN[1], G. BABKIN[1], A. PINHASOV[2];
[1]Lactocore Inc., Plymouth, MI; [2]Dept. of Mol. Biol. and Adelson Sch. of Med., Ariel Univ., Ariel, Israel

Abstract: Background. LCGA-17 is a novel peptide with potent anxiolytic and antidepressant properties that acts through α2δ, with possible synergism by $GABA_A$ receptors. The aim of the current study was to evaluate the effects of LCGA-17 in socially dominant (Dom) and socially submissive (Sub) mice. These two populations had been selectively bred from a background Sabra strain for the behavioral traits of dominance and submissiveness. Dom mice are stress-resilient, exhibiting manic-like behavior and paradoxical effect to the anxiolytic and antidepressant agents. In contrast, Sub mice are stress vulnerable and exhibit depressive-like and impaired social features. **Methods.** LCGA-17 was administered in doses of 0.1, 0.3, and 1 mg/kg intranasally for 10 days. The Three Chamber Test (TChT) was carried out on day 7 and the Hole Board (HB) test was carried out on day 10 of the treatment. Diazepam (Diaz) was used as a reference drug. After the behavioral experiments' prefrontal cortexes (PFC), and hippocampi (Hip) of the mice were dissected. Analysis of elongation factor 2 kinase (EF2K) as well as phosphorylated elongation factor EF2(T56) were carried out with qPCR and WB. **Results.** Dom mice treated with LCGA-17 displayed dose-dependent novelty-seeking behavior with greater sociability in the TChT, a similar but less pronounced effect was also observed in Sub. In the HB, both Diaz and LCGA-17produced an increment of head-dipping in Sub mice, suggesting an anxiolytic-like effect of the peptide in stress-vulnerable population. In Dom mice, Diaz failed to alter this parameter, while LCGA-17 increased head-dipping, which may suggest a potential antipsychotic effect in animals with manic-like phenotype. Chronic LCGA-17 treatment (1mg/kg) increased EF2K protein level as well as EF2(T56) phosphorylation in Dom mice in both PFC and Hip. In Sub mice, we observed an induction of EF2(T56) in Hip only. EF2K inactivates EF2 by phosphorylation on threonine 56 (T56), which results in the inhibition of protein synthesis. **Conclusion.** The results in the TChT suggest a potential implication of LCGA-17 in social phobia treatment. The reduction of protein translation in mouse brains might be implicated in the disruption of the process of traumatic memory consolidation and may diminish PTSD symptoms. This mechanism could underlie LCGA-17 anxiolytic action. Thus, these results suggest a selective anxiolytic/antidepressant-like or mood-stabilizing effect of LCGA-17 in socially submissive and socially dominant populations, respectively.

Disclosures: A. Malyshev: A. Employment/Salary (full or part-time):; full-time employment at Lactocore Inc. **E. Shmerkin:** 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.; principal investigator for a drug stud. **A. Klinov:** 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.; investigator for a drug study for Lactocore Inc. **L. Vinnikova:** 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.; investigator for a drug study. **D. Begmatova:** 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.; investigator for a drug study for Lactocore Inc. **D. Sur:** 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.; principal investigator for a drug study for Lactocore Inc. **N. Mitkin:** A. Employment/Salary (full or part-time):; full-time employment at Lactocore Inc. **I. Sukhanova:** A. Employment/Salary (full or part-time):; full-time employment at Lactocore Inc. **V. Gedzun:** A. Employment/Salary (full or part-time):; full-time employment at Lactocore Inc. **V. Pavshintsev:** A. Employment/Salary (full or part-time):; full-time employment at Lactocore Inc. **I. Doronin:** A. Employment/Salary (full or part-time):; full-time employment at Lactocore Inc. **G. Babkin:** A. Employment/Salary (full or part-time):; full-time employment at Lactocore Inc. **A. Pinhasov:** 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.; consultant for Lactocore Inc..

Poster

P187. Brain Injury and Wellness: Pharmacological Interventions

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P187.02

Topic: C.01. Brain Wellness and Aging

Support: DOD Award # W81XWH1910702

Title: Long-term Changes in Neuroimaging Markers, Cognitive Function and Psychiatric Symptoms in an Experimental Model of Gulf War Illness

Authors: *X. WU[1], A. K. SHETTY[2], D. S. REDDY[1];
[1]Neurosci. and Exptl. Therapeut., [2]Mol. and Cell. Med., Texas A&M Univ. Hlth. Sci. Ctr., Bryan, TX

Abstract: Gulf War Illness (GWI) is a multi-symptom disease with debilitating cognitive and emotional impairments in veterans. GWI is caused by chemical neurotoxicity and manifests from disturbances in central and peripheral network excitability. However, the mechanisms underlying such devastating neurological and psychiatric symptoms remain unclear. Here we investigated the long-term changes in neurological behavior and brain neuroimaging abnormalities in the GWI-like model of chemical neurotoxicity in rats. GWI is linked to exposure to GWI-related organophosphate chemicals (PB, DEET, and permethrin) during the stressful Gulf war. Experimental GWI was induced in rats by 4 weeks of daily exposure to GWI related chemicals with stress restrain (GWIR-CS). The cohorts were screened in the novel object recognition memory test, open-field test, elevated plus-maze test, and social interaction test. At 5-months and 10-months post-GWIR-CS, rats exhibited significant signs of memory and emotional impairment. Exposure to GWIR-CS produced significant decrease in time spent with the novel object, time spent in the central zone in the open-field, time spent on open arms and entries in the

elevated plus-maze at 5-mon and 10-mon post-GWIR-CS. In forced swimming test, the rats showed significant decreased interests in swimming. These data were confirming strong deficits in emotion and anxiety behaviors. They displayed heightened aggression and reduced social interactive time, indicating stressful anxiety and mood disturbances. In addition, significant changes were evident in learning and memory performances in acquisition and recall of spatial memory in the Morris water-maze test. In the MRI scanning, there were significant increases in lateral ventricle T2 relaxation times and significant differences in the volumes of hippocampus, thalamus, and lateral ventricle at 10 months. These results confirm that chronic GWIR-CS exposure led to striking cognitive and psychiatric deficits along with brain tissue lesions, demonstrating the potential utility of this model for therapeutic interventions for GWI and its comorbidities.

Disclosures: X. wu: None. **A.K. Shetty:** None. **D.S. Reddy:** None.

Poster

P187. Brain Injury and Wellness: Pharmacological Interventions

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P187.03

Topic: C.01. Brain Wellness and Aging

Support: AHA Grant 33700076
NIH Grant R01NS112642

Title: Cofilin knockdown improved behavioral and cognitive functions in ICH aged male and female mice

Authors: *D. ALMARGHALANI, Z. SHAH;
The Univ. of Toledo, Toledo, OH

Abstract: Intracerebral hemorrhage (ICH) represents only 10-15% of total stroke types but carries a high risk of morbidity and mortality, and survivors suffer from long-term disabilities and post-stroke cognitive impairments (PSCI). The overall mortality rate in aged individuals is higher than that of younger individuals. However, the mechanisms involved in secondary injury-induced PSCI after ICH is not clear yet. Our lab reported that inhibiting cofilin with small interfering RNA (siRNA) 72-h prior to induced ICH improved secondary injury-induced neurobehavioral deficits and reduced hemorrhagic volume and microglia activation in mice. In the present study, we investigated if cofilin siRNA treatment immediately after ICH will mitigate the secondary injury and enhance PSCI in aged (18-22 months) male and female mice. The animals were survived for 15 days to observe therapeutic effects. Neurobehavioral deficits and grip strength were performed before (baseline) and after ICH on days 1, 3, 5, 7,10, and 15. Both male and female mice suffered from neurobehavioral deficits after ICH, which was mitigated by

cofilin siRNA treatment. Following the ICH, mice exhibited a sustained functional deficiency of the forelimb strength which was improved with cofilin siRNA treatment. The cognitive function assessed by Y-maze (spatial memory) at 11, 12, 13, and 14 days showed improvement with cofilin siRNA treatment in both male and female mice. These results suggest that siRNA-based gene therapy presents a viable treatment option for ICH.

Disclosures: D. Almarghalani: None. **Z. Shah:** None.

Poster

P187. Brain Injury and Wellness: Pharmacological Interventions

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P187.04

Topic: C.01. Brain Wellness and Aging

Support: Cook Medical Advances in Medicine Award (NIH, NCATS, CTSA)

Title: Raloxifene decreases lumbar intervertebral disc degeneration and increases rewarding corn syrup intake in aged female mice.

Authors: *O. EL JORDI[1], N. BHADOURIA[2], D. L. MCKINZIE[1], N. HOLGUIN[3]; [1]Pharmacol. & Toxicology, Indiana Univ. Sch. of Med., Indianapolis, IN; [2]Sch. of Mechanical Engin., Purdue Univ., West Lafayette, IN; [3]Dept. of Mechanical and Energy Engin., Indiana University-Purdue Univ., Indianapolis, IN

Abstract: Among various bone-related consequences, osteoporosis elevates the risk for vertebral fractures and can promote intervertebral disc (IVD) degeneration that results in chronic back pain and adversely affects activities of daily living. Raloxifene, a selective estrogen receptor modulator, is indicated for osteoporosis in postmenopausal women and has therapeutic benefit in reducing both vertebral fractures and, for unclear reasons, providing symptomatic relief of back pain. Using aged female C57BL/6J mice as an animal model of age-related IVD degeneration, we hypothesized that raloxifene would attenuate IVD pathology and improve physical and psychological measures of health. Mice (n=8/group) were treated for 6 weeks with single daily injections (Monday-Friday dosing) of either raloxifene (0.5 mg/kg) or vehicle. Both prior to and following treatment, mice were evaluated for grip strength, hind-limb clasping, open-field locomotor activity in a light-dark preference test, and consumption of a highly palatable corn syrup solution. Treatment, food and water intake, and wheel running behavior were monitored continuously using home-cage telemetric recording. Following treatment and behavioral assessments, mice were necropsied and spinal degeneration quantified using a computed total IVD degeneration score and section-wise score (nucleus pulposus/annulus fibrosus) of Safranin-O stained motion segments. Additionally, pain biomarker Substance P expression was quantified by immunohistochemistry. Pathology results found a significant reduction in total IVD and

section-wise degeneration scores ($p<0.05$, between-group t-test) in mice treated with raloxifene relative to vehicle treatment. Moreover, raloxifene treatment resulted in significantly reduced Substance P expression levels. Interestingly, the most pronounced behavioral assessment that detected an effect of raloxifene was intake of the highly palatable corn syrup solution. While both groups drank little water when provided with an overnight access to corn syrup, raloxifene-treated mice drank 20% more corn syrup solution than controls. The selective effect of raloxifene treatment on this palatability drinking measure of hedonia suggests potential therapeutic benefit in improving affective domains impacted by IVD degeneration and warrant further investigation.

Disclosures: O. El Jordi: None. **N. Bhadouria:** None. **D.L. McKinzie:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Eli Lilly & company shareholder. **N. Holguin:** None.

Poster

P187. Brain Injury and Wellness: Pharmacological Interventions

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P187.05

Topic: C.01. Brain Wellness and Aging

Support: NIH/NIA T32 AG 020494
UNTHSC Seed Grant

Title: The neurobehavioral and neurochemical effects of early, chronic methamphetamine exposure in adult and middle-aged mice

Authors: *D. L. DAVIS, P. H. VANN, J. M. WONG, D. B. METZGER, R. A. SHETTY, M. J. FORSTER, N. SUMIEN;
Pharmacol. and Neurosci., Univ. of North Texas Hlth. Sci. Ctr., Fort Worth, TX

Abstract: Prescription stimulant misuse has increased over the last decade, with amphetamine-type stimulants becoming the second most used drug by American college students. Misuse of these psychostimulant drugs can lead to serious health effects linked to their high abuse liability and potential for neurotoxicity. Even at moderate doses, psychostimulants can increase cellular oxidative stress via auto-oxidation or metabolism of dopamine. Heightened levels of oxidative stress have been associated with neurobehavioral impairments and dopaminergic pathways are particularly vulnerable to oxidative insults. We hypothesize that oxidative stress may act as a prominent cellular mechanism in mediating psychostimulant-induced neurotoxicity and associated neurobehavioral deficits. Our study examined the short and long-term consequences of chronic administration of the prototypical psychostimulant, methamphetamine (METH), on motor and cognitive function and markers of oxidative stress, dopaminergic and glial function. Groups of 4 month old young male and female (short-term group, n = 11-12; long-term group, n

= 19-20) C57BL/6J mice received non-contingent intraperitoneal injections of either saline or methamphetamine (1.4 mg/kg) twice daily for 4 weeks (Mon-Fri). The short-term (ST) cohorts were behaviorally tested 2 weeks after the last injection and the long-term (LT) cohorts, were tested every 2 months for motor function and for cognition 4 months after the last injection. Behavioral tests were: the Morris water maze, active avoidance, and fear conditioning, bridge walking coordinated running and gait analyses. After behavioral testing, the mice were euthanized and the brain was further processed to measure oxidative stress, dopaminergic function (tyrosine hydroxylase (TH); Dopamine Transporter (DAT)), and glial function (GFAP). Our behavioral outcomes revealed cognitive deficits in the short-term cohorts, with no long-lasting effect in the long-term cohorts. Longitudinal evaluation of motor function supported motor deficits in gait and coordinated running, while bridge walking and spontaneous activity were not affected. TH and oxidative stress were not greatly affected in both ST and LT groups, while DAT was increased in the ST and marginally in the LT cohort. GFAP, especially the caspase 6-mediated cleavage product, was affected in both ST and LT cohorts. These findings do not support our hypothesis that oxidative stress played a major role in METH-induced impairments of motor and cognitive function. These data indicate that dopaminergic pathway disturbance and astrocytes may play a role in these observed neurobehavioral impairments.

Disclosures: D.L. Davis: None. **P.H. Vann:** None. **J.M. Wong:** None. **D.B. Metzger:** None. **R.A. Shetty:** None. **M.J. Forster:** None. **N. Sumien:** None.

Poster

P187. Brain Injury and Wellness: Pharmacological Interventions

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P187.06

Topic: C.01. Brain Wellness and Aging

Support: Cedars-Sinai Center for Research in Women's Health and Sex Differences
Cedars-Sinai Board of Governors Regenerative Medicine Institute

Title: iPSC-derived immune cells have a regenerative effect in mouse models of aging

Authors: *V. A. MOSER, R. M. LIPMAN, M. J. WORKMAN, C. N. SVENDSEN;
Board of Governors Regenerative Med. Inst., Cedars-Sinai Med. Ctr., West Hollywood, CA

Abstract: Previous studies have demonstrated the ability of young blood or plasma to restore cognitive function in aged animals and our lab has shown beneficial effects of young mouse-derived bone marrow transplants into aged mice. However, both of these strategies have significant practical drawbacks that limit their therapeutic value. Induced pluripotent stem cells (iPSCs) offer the ability to generate an autologous therapy. Thus, we sought to identify the cell type responsible for the beneficial effects observed in studies using young plasma and bone

marrow. We generated macrophages from iPSCs (iMACs), which express low levels of the hematopoietic stem cell marker CD34, and high levels of the monocyte/macrophage markers CD11b, CD14, and CD16. iMACs were administered to aged, genetically immunocompromised NOD-scid-gamma (NSG) mice via tail vein injection. Mice received treatments every 3rd day for 22 days and were tested on a number of behavioral assays. We find significant improvements in spatial working memory in the spontaneous alternation test as well as in hippocampal-dependent short-term memory in the novel object placement assay. Treatment with iMACs has significant effects on several key neuronal health indices including the synaptic transporter, VGLUT1, which is decreased in aged mice but restored with treatment. Moreover, the increased numbers of astrocyte and microglia as well as the decrease in microglial branch length seen in aged animals are all reversed by treatment with iMACs. These findings suggest that immune cells, specifically monocytes and macrophages, may be responsible for the regenerative effects previously observed after young plasma transfusions or bone marrow transplants. Importantly, we find that iMACs have significant regenerative potential in aging.

Disclosures: V.A. Moser: None. **R.M. Lipman:** None. **M.J. Workman:** None. **C.N. Svendsen:** None.

Poster

P187. Brain Injury and Wellness: Pharmacological Interventions

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P187.07

Topic: C.01. Brain Wellness and Aging

Title: Pharmacokinetics and distribution of AKST4290, a CCR3 antagonist with anti-inflammatory properties in the eye and brain

Authors: *S. RAINA, R. HARISH, Y. LOPEZ, S. REGE, J. MASUMI, A. TEICHERT, O. S. DHANDE, S. S. MINAMI;
Alkahest, San Carlos, CA

Abstract: Age-related macular degeneration (AMD) is an inflammation driven degenerative disease and can lead to severe vision loss. After receiving 6 weeks of orally dosed AKST4290, a potent CCR3 inhibitor, the majority of neovascular AMD (nAMD) patients in phase 2a trials showed stable or improved best-corrected visual acuity. We used the sodium iodate model to investigate the effects of CCR3 inhibition on retinal inflammation. CCR3 inhibition significantly reduced CXCL9 and CCL5, potent immune cell chemoattractants, in the eye. This suggests that inhibition of the CCR3-mediated immune response may reduce ocular inflammation, which in turn may contribute to the efficacy of AKST4290 observed in nAMD patients.
To identify the potential sites of action, pharmacokinetic studies were conducted in the eye, as well as the brain. Autoradiography (ARG) and quantitative whole-body autoradiography

(QWBA) analysis showed preferential retention of orally dosed AKST4290 in the uveal tract in both male and female mice. To localize the retention of AKST4290 in the eye, different retinal compartments were analyzed using liquid chromatography with tandem mass spectrometry (LC-MS/MS) and AKST4290 was found to be retained at higher levels in the retinal pigmental epithelium (RPE)/choroid compared to the retina. QWBA and LC-MS/MS was also used to assess the impact of pigmentation on drug concentration and showed that there were increased levels of the drug in pigmented RPE versus non-pigmented RPE. Additionally, AKST4290 has also been shown to reduce neuroinflammation in the brain. QWBA was used to assess the site of action in the brain and showed no AKST4290 present. LC-MS/MS analysis also showed very low levels in the brain, together demonstrating that it is non-brain penetrant drug. These data suggest that AKST4290 treatment for nAMD can act both systemically as well as centrally in the RPE/choroid, where action may occur through inflammatory pathways.

Disclosures: S. Raina: A. Employment/Salary (full or part-time):; Grifols. **R. Harish:** A. Employment/Salary (full or part-time):; Grifols. **Y. Lopez:** A. Employment/Salary (full or part-time):; Grifols. **S. Rege:** A. Employment/Salary (full or part-time):; Grifols. **J. Masumi:** A. Employment/Salary (full or part-time):; Grifols. **A. Teichert:** A. Employment/Salary (full or part-time):; Grifols. **O.S. Dhande:** A. Employment/Salary (full or part-time):; Grifols. **S.S. Minami:** A. Employment/Salary (full or part-time):; Grifols.

Poster

P270. Human Studies: Genetics and Diagnostics PD I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P270.01

Topic: C.03. Parkinson’s Disease

Title: Zenchi - a VR-based remote patient monitoring system for diagnosis of obstructive sleep apnea in Parkinson's disease patients

Authors: *J. ALZAGATITI, K. AJJARAPU, K. PANDRANGI;
Univ. of California Santa Barbara, Isla Vista, CA

Abstract: Parkinson’s disease (PD) is the second most common neurodegenerative disease. It is characterized by motor and non-motor features, one of which is obstructive sleep apnea (OSA). OSA is reported in 20-60% of PD patients and is diagnosed through polysomnography at a sleep center. However, due to COVID-19 restrictions to in-person care, telehealth monitoring of sleep data to detect OSA is a key alternative. With current telehealth, physicians experience tremendous difficulty accessing patient vitals, such as sleep data, and complex reports, such as CT scans and MRIs. To address this problem, we developed Zenchi, a VR-based remote patient monitoring system. Zenchi enables physicians to view the patient's real-time vitals, e.g. O^2 saturation, heart rate, and sleep data; while also displaying 3D patient reports in an integrated

view, improving the quality of care. Patients are able to use wearables and other devices at home to efficiently send necessary data within Zenchi for physicians to use on demand. Our system can not only identify and monitor OSA within the totality of a patient's medical records, but also help centralize data for physicians for any health issue.

Disclosures: J. Alzagatiti: None. **K. Ajjarapu:** None. **K. Pandrangi:** None.

Poster

P270. Human Studies: Genetics and Diagnostics PD I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P270.02

Topic: C.03. Parkinson's Disease

Title: Key Risk Pathways in Aging and Parkinson's Disease Pathogenesis Revealed by Mulitomic Single-nuclei analysis of Human Young, Aged, and PD Midbrain Samples

Authors: *L. ADAMS[1], M. SONG[1], Y. TANAKA[2], Y.-S. KIM[1];
[1]RWJMS Inst. for Neurolog. Therapeut., Piscataway, NJ; [2]Dept. of Med., Univ. of Montreal, Montreal, QC, Canada

Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disease, characterized by the loss of dopamine neurons from the substantia nigra (SN). While many genetic components and environmental risk factors have been identified, aging is the primary risk factor for PD although it remains unclear how aging predisposes an individual to neurodegenerative conditions. In this study, we analyzed 71,106 SN nuclei from 32 individuals to compare young, aged, and PD-diagnosed conditions. We analyzed gene expression and chromatin accessibility simultaneously at the single-nuclei level using paired snRNA-seq and snATAC-seq to identify aging and disease-specific changes in the human midbrain. We identified seven cell types, with oligodendrocytes (OL) making up approximately 75% of the cells in the midbrain. Our data shows widespread, cell type specific changes in chromatin states over aging that influence gene expression. To investigate the contribution of aging to PD pathogenesis, we adapted pseudotime frameworks to combine RNA and ATAC data and established a pseudopathogenesis trajectory between young, old and PD conditions. This analysis allowed us to identify aging and disease relevant cell type populations, their gene expression patterns and chromatin motifs, and compare them with existing GWAS and SNP databases. Astrocytes and oligodendrocyte precursor cells show significant alterations over aging, but no significant differences during PD pathogenesis, suggesting they have little contribution to PD. Interestingly, although dopamine neurons are sparsely myelinated, OL show dramatic changes both over aging and PD. Pseudopathogenesis analysis in OL indicates that genes involved in myelination-related pathways are gradually decreased over aging toward PD, while stress response-related genes are increased. Our multiomic analysis of single-cell transcriptomic and

epigenomic dataset provides an essential resource to further our understanding of how aging affects the midbrain and predisposes this region to degeneration in PD.

Disclosures: L. Adams: None. **M. Song:** None. **Y. Tanaka:** None. **Y. Kim:** None.

Poster

P270. Human Studies: Genetics and Diagnostics PD I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P270.03

Topic: C.03. Parkinson's Disease

Support: This work was supported by grant from the NIH/NINDS NS38377 Morris K. Udall Parkinson's disease Research Center.

Title: Evaluating the Biomarker Potential of c-Abl Pathway Molecules using Serum Derived Exosomes

Authors: *D. BISWAS[1], S. BRAHMACHARI[1], R. SHI[1], W. RANKIN[1], L. ROSENTHAL[2], V. L. DAWSON[1], T. M. DAWSON[1];
[1]Neuroregeneration and Stem Cell Programs, Inst. for Cell Engineering, Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [2]Dept. of Neurology, Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: The complexities of Parkinson's disease (PD) pathogenesis have made it difficult to produce reliable biomarkers that reflect disease diagnosis. Therefore, discovering molecules that can both be isolated non-invasively and indicate disease severity has significant clinical application. We earlier established that activated c-Abl, a nonreceptor tyrosine kinase (pY245 c-Abl), phosphorylates α-synuclein at tyrosine-39 (pY39 α-synuclein) and both pY245 c-Abl and pY39 α-synuclein levels are profoundly elevated in animal models of PD as well as postmortem PD brains. c-Abl also phosphorylates parkin, an E3 ubiquitin ligase, at tyrosine-143. This inactivates parkin and leads to accumulation of pathogenic parkin substrates such as PARIS (ZNF746) and AIMP2, both of which are also significantly elevated in PD mouse models and postmortem PD brains. As such, we hypothesize that these molecules are elevated in serum-derived exosomes and could serve as blood-based biomarkers for PD. Exosomes are vesicular structures released by all cells and contain proteins and nucleic acids. Particularly, exosomes containing L1CAM, a transmembrane protein preferentially expressed on neurons, was reported as a potential diagnostic tool for neurodegenerative diseases. To determine if L1CAM+ exosomes reflect PD biology, we used a double-blind setup to isolate human serum exosomes from PD patients and age-matched healthy controls in two independent cohorts: Johns Hopkins Parkinson's Disease Biomarkers Program (n=106), and Cleveland Clinic (n=50). L1CAM+ exosomes were obtained with Exoquick™, a nanoparticle-based exosome isolation method,

followed by an enrichment process using L1CAM antibodies. Although L1CAM is preferentially expressed in neurons, it is also found in many other cell types. As a brain-specific window for serum exosomes is not available, we also isolated total serum exosomes from PD patients and age-matched healthy controls from Johns Hopkins Udall Center cohorts (n=66) using a high-throughput size-exclusion-based exosome isolation kit. The levels of pY245 c-Abl, pY39 α-synuclein, α-synuclein, PARIS, and AIMP2 in exosome lysates were determined with immunoblot assays and Meso Scale Discovery (MSD) ELISA assays. The results from immunoblot assays show that pY245 c-Abl, pY39 α-synuclein, PARIS, and AIMP2 levels are significantly elevated in both L1CAM and total serum-derived exosomes among all 3 independent patient cohorts. We further confirm our immunoblot results using quantitative MSD ELISA assays. Taken together, our findings strongly suggest that c-Abl pathway molecules can potentially serve as blood-based diagnostic markers of PD.

Disclosures: D. Biswas: None. **S. Brahmachari:** None. **R. Shi:** None. **W. Rankin:** None. **L. Rosenthal:** None. **V.L. Dawson:** None. **T.M. Dawson:** None.

Poster

P270. Human Studies: Genetics and Diagnostics PD I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P270.04

Topic: C.03. Parkinson's Disease

Support: Beca postdoctoral Fundacion Ramon Areces
JPB Grant

Title: Chaperone-mediated autophagy as a novel therapeutic target in Parkinson's disease

Authors: *A. MARTIN-SEGURA[1], K. TEKIRDAG[1], R. GOMEZ-SINTES[2], M. BOURDENX[1], T.-I. KAM[3], L. FAN[4], S. K. SEE[5], J. MADERO-PÉREZ[4], T. M. DAWSON[3], V. L. DAWSON[3], D. L. SULZER[6], L. GAN[4], M. KAMPMANN[5], A. CUERVO[1];
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Abstract: Proteostasis or protein homeostasis is required by living cells to maintain a functional proteome. Enhanced proteostasis has been related to increased life- and health- span, while proteostasis loss has been described in aging and age-related diseases. In particular, the loss of proteostasis is one of the most common features in neurodegenerative diseases such as Alzheimer's and Parkinson's disease (PD), where different proteins form aggregates that directly affect the progression of the disease and the neuronal survival. Our project focuses on the role

that malfunctioning of a component of the proteostasis network, Chaperone-mediated autophagy (CMA), plays in the pathogenesis of PD. PD is the most common motor disorder in the elderly and it is characterized by severe motor symptoms and dementia. Our group has previously stablished *in vitro* the toxic effect that different PD-related pathogenic proteins such as α-Synuclein or LRRK2 exert on CMA. These CMA alterations in turn could contribute to neurodegeneration and PD progression. In this work, using different mouse models of PD and patient brains, we demonstrate *in vivo* the toxic effect of pathogenic α-Synuclein on CMA activity. We also show how CMA failure can accelerate PD progression and demonstrate the beneficial effect of enhancing CMA activity on PD models. Our results have encouraged us to develop new strategies to overcome the toxic effect of α-Synuclein on CMA. To that effect, we have conducted a genome-wide CRISPR interference screening in cells expressing a pathogenic form of a-Synuclein and a fluorescent reporter for CMA. We anticipate that the newly identified CMA regulators will provide novel targets to modulate CMA with therapeutic purposes in PD.

Disclosures: A. Martin-Segura: None. **K. Tekirdag:** None. **R. Gomez-Sintes:** None. **M. Bourdenx:** None. **T. Kam:** None. **L. Fan:** None. **S.K. See:** None. **J. Madero-Pérez:** None. **T.M. Dawson:** None. **V.L. Dawson:** None. **D.L. Sulzer:** None. **L. Gan:** None. **M. Kampmann:** None. **A. Cuervo:** None.

Poster

P270. Human Studies: Genetics and Diagnostics PD I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P270.05

Topic: C.03. Parkinson's Disease

Support: This research was supported by the Intramural Research Programs of the NINDS and NHGRH.

Title: Imaging and Genetics: Effects of GBA gene on Parkinson's Disease

Authors: *S. GHATTI[1], E. YOON[1], G. LOPEZ[2], D. EHRLICH[1], S. G. HOROVITZ[1]; [1]Natl. Inst. of Neurolog. Dis. and Stroke, Bethesda, MD; [2]Natl. Human Genome Res. Inst., Bethesda, MD

Abstract: Parkinson's Disease (PD) is a common neurodegenerative disorder that is characterized by motor and nonmotor symptoms. A number of genetic variants have been associated with an increased risk for developing PD, such as variants in *GBA*, where specific variants have been associated with faster disease progression. Given these phenotypic changes associated with *GBA* variants, we aimed to explore whether cortical thickness and other markers of neurodegeneration differed in healthy controls and patients with Parkinson's disease with and without *GBA* variants.

Neuroimaging and biospecimen data were obtained from the Parkinson's Progression Markers Initiative (PPMI) database. Using FreeSurfer, we compared T1-weighted MRI images from 47 healthy controls to those of 94 PD patients. The PD cohort included subjects with heterozygousvariants in N370S (N=21), E326K (N=18), T369M variant (N=8) and non-variant carriers (N=47). After an FDR correction of 0.01, we found significant differences between controls and PD patients in ROIs in the following areas: the lateral occipital, superior parietal, and postcentral regions. Within these ROIs, we then compared cortical thickness of each PD group to the control, while accounting for age and ethnicity. Cortical thickness of all PD patients differs fromcontrols in the parieto-occipital areas, where individuals with E326K and T369M variants, aswell as non-variant carriers, showed stronger effects than those with N370S variant. In addition,we compared biospecimen data between the PD groups. There were no significant differences in CSF Abeta, p-tau, and t-tau levels amongst the groups. N370S carriers had significantly higher serum neurofilament light (NfL) levels, a measure of cortical degeneration, among all groups.Our results demonstrate significant cortical thickness changes in PD patients when compared tocontrol group. They also highlight the impact of *GBA* variant on the PD presentation in the parieto-occipital cortex. Finally, they suggest that PD patients with N370S variants have a different disease presentation than PD patients with variants on the same *GBA* gene.

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Poster

P270. Human Studies: Genetics and Diagnostics PD I

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Program #/Poster #: P270.06

Topic: C.03. Parkinson's Disease

Support: NIA F30AG067651
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Michael J. Fox Foundation for Parkinson's Research
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Alzheimer's Research UK
The Weston Brain Institute

Title: Subcortical iron accumulation pattern may predict neuropsychological outcomes after STN DBS: a pilot study

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Abstract: Background: Neuropsychological outcomes after deep brain stimulation (DBS) are variable and may arise from the heterogeneous neuropathological processes in Parkinson's disease (PD).
Objective: To explore if brain iron accumulation patterns and its region-specific alterations relate to neuropsychological outcomes post-DBS.
Methods: Thirty-two PD subjects were identified from our database with susceptibility MRI prior to bilateral subthalamic nucleus (STN) DBS between 2011-2016. Demographic (age, sex, education), clinical information (disease duration, neuropsychological scores), and R2* (susceptibility MRI measure reflecting iron) in 11 subcortical regions of interest were obtained. Neuropsychological outcomes were defined as changes in psychomotor speed, executive function, attention, memory, and depression by subtracting pre- and post-DBS scores. A penalized logistic analysis was used to identify the best pre-DBS clinical and R2* predictors for each neuropsychological domain. Pearson's partial correlations explored R2* associations with neuropsychological outcomes.
Results: Combined clinical and MRI metrics were associated better with neuropsychological outcomes ($R^2 \geq 0.373$, p-value≤ 0.008) than either alone. Adding R2* metrics increased prediction of executive function ($R^2=0.455$, $p=0.008$) and attention ($R^2=0.182$, $p=0.018$) outcomes over clinical metrics alone. Specifically, R2* in the substantia nigra, caudate, STN, and hippocampus improved prediction of executive function, and in the putamen for attention. Interestingly, higher caudate R2* correlated with better executive function ($p=0.043$), whereas higher putamen R2* associated with worsening attention ($p=0.018$).
Conclusions: Brain iron accumulation patterns, captured by susceptibility MRI, may add value to clinical evaluation in predicting neuropsychological outcomes post-DBS in PD. Further studies are warranted to validate these findings and understand the region-specific relationships between iron and DBS outcomes.

Disclosures: G. Brown: None. **G. Du:** None. **E. Farace:** None. **M.M. Lewis:** None. **P. Eslinger:** None. **J. McInerney:** None. **L. Kong:** None. **R. Li:** None. **X. Huang:** None. **S. De Jesus:** None.

Poster

P391. Pain Models: Pharmacology II

Location: Virtual Only

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Program #/Poster #: P391.01

Topic: D.03. Somatosensation – Pain

Title: (2r,6r)-hydroxynorketamine fails to produce analgesia in assays of pain-stimulated and pain-depressed behaviors in mice.

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Abstract: Ketamine has been shown to produce analgesia in various acute and chronic pain states; however, abuse liability concerns have limited its utility. The ketamine metabolite (2R,6R)-hydroxynorketamine ((2R,6R)-HNK) produces antidepressant-like effects similar to ketamine without the abuse liability concerns. (2R,6R)-HNK produces sustained analgesia in models of chronic pain but has yet to be evaluated in models of acute pain. The present study evaluated the efficacy of (2R,6R)-HNK in assays of pain-stimulated (hot plate test and acetic acid writhing) and pain-depressed behavior (locomotor activity and rearing). In assays of pain-stimulated behaviors, (2R,6R)-HNK (1-32 mg/kg) failed to produce antinociception in the hot plate (52- and 56-degree) and acetic acid writhing assays. In assays of pain-depressed behaviors, 0.56% acetic acid produced a robust depression of locomotor activity and rearing that was not blocked by pretreatment of (2R,6R)-HNK (3.2-32 mg/kg). The positive controls morphine (hot plate test) and ketoprofen (acetic acid writhing, locomotor activity, and rearing) blocked pain-stimulated and pain-depressed behaviors. These results suggest that (2R,6R)-HNK is unlikely to have efficacy in treating acute pain; however, the efficacy of (2R,6R)-HNK in chronic pain states should continue to be evaluated.

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Poster

P391. Pain Models: Pharmacology II

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Program #/Poster #: P391.02

Topic: D.03. Somatosensation – Pain

Support: NIDCR/NIH R56DE025298
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T32 GM133352-02

Title: Tissue-specific regulation of miR-374a-5p and its mRNA targets associated with pain in humans and mice

Authors: *N. HERNANDEZ[1,2], A. RAWLS[1], Y. WANG[2], X. ZHANG[2], J. CHEN[2], Y. PAN[3], H. DANG[4], G. SLADE[5], S. LINNSTAEDT[5], M. PARISIEN[6], L. DIATCHENKO[6], A. NACKLEY[2,1];
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Abstract: MicroRNAs (miRNAs) are of growing importance to the understanding of pain neurobiology. To identify miRNAs contributing to pain, our group evaluated expression of miRNAs and respective mRNA targets in cohorts with chronic and first-onset pain: one with chronic temporomandibular disorder (TMD) (N= 488) and one with acute pain following a motor vehicle collision (MVC) (N= 167). We found that miR-374a-5p was downregulated in both male and female patients with TMD and MVC-related pain compared to controls. Further, miR-374a-5p was downregulated in our pre-clinical mouse model of acute stress and pain (N= 31). This work provided the first link between miR-374a-5p and pain. What remains unknown is the tissue-specific regulatory effects of circulating miR-374a-5p on its mRNA targets. Thus, the purpose of the present study was to measure expression of miR-374a-5p's mRNA targets associated with clinical pain in tissues collected from mice undergoing stress and pain. Of the 350 mRNA targets upregulated in patients with TMD, we selected 10 (ATXN7, CBL, CRK, CRKL, EP300, HIF1A, KIDINS220, NUMB, PTPN11, TGFBR2) to measure in our mouse model based on: strength of association with miR-374a-5p, magnitude of fold-change in TMD cases compared to controls, and gene function implications. Wildtype male and female C57BL6 mice ~8-12 weeks old were exposed to forced swim stress or sham swim for 3 consecutive days and then, on day 3 following swim, they received an i.p. injection of the COMT inhibitor OR486 or vehicle (N= 4M+4F x 4 groups = 32 mice). Tissues (leukocytes, intrascapular brown adipose, visceral white adipose, subcutaneous white adipose, dorsal root ganglion, and spinal cord) were collected 1 hour following OR486 administration. Relative mRNA expression across conditions was determined by qPCR and 2-ΔΔCt analysis. Differential expression of targets was observed across multiple tissues. Notably, mice with swim stress and COMT inhibition showed increased levels of HIF1A (Hypoxia Inducible Factor 1 Subunit Alpha; stress response), NUMB (NUMB Endocytic Adaptor Protein; neurogenesis), and PTPN11 (Protein Tyrosine Phosphatase Non-Receptor Type 11; cell signaling) in adipose and spinal tissues, with higher levels in females compared to males. This set of correlations provides a novel framework for future experiments to confirm miRNA-mRNA interactions and determine the causal role of miR-374a-5p in the onset and maintenance of pain. Additionally, this work provides rationale for examining specific tissues and sex differences in pain neurobiology.

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Poster

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Program #/Poster #: P391.03

Topic: D.03. Somatosensation – Pain

Support: Conacyt Grant A-S1-40015

Title: Rage and tlr5 receptors contribute to nociception in a rat model of fibromyalgia

Authors: *L. E. VILLANUEVA-SOLIS, V. GRANADOS-SOTO;
Pharmacobiology, CINVESTAV, Tlalpan, Mexico

Abstract: Fibromyalgia is characterized by the presence of widespread musculoskeletal pain for which there is no defined cause. Hypersensitivity to pain produced in patients with fibromyalgia could be derived from a dysfunction of the immune system. Evidence suggest that an imbalance of pro- and anti-inflammatory cytokines could lead to the development of hypersensitivity and other co-morbidities in this syndrome. Higher levels of interleucine-8 (IL-8), tumoral necrosis factor (TNF) and high mobility group box-1 (HMGB1) has been found in serum and cerebrospinal fluid of these patients. HMGB1, through the activation of spinal and peripheric Toll-like receptors (TLRs) and advanced glycosylation end products receptor (RAGE) facilitates transmission of pain in models of inflammatory and neuropathic pain. However, the role of TLR5 and RAGE receptors in functional pain is unknown. The purpose of this study was to determine the involvement of TLR5 and RAGE receptors in a reserpine-induced model of fibromyalgia in rats. Subcutaneous administration of reserpine (1 mg/kg, 3 days) in female Wistar rats produced generalized tactile allodynia and muscle hyperalgesia at 5 and 7 days after the last injection, respectively. Von Frey filaments were used to determine tactile allodynia and the Randall-Selitto test was used to assess muscle hyperalgesia. Reserpine-treated rats were intrathecally injected with the TLR5 selective inhibitor TH1020 (30-300 nmol) or the RAGE selective antagonist FPS-ZM1 (1-100 nmol). Intrathecal injection of TH1020 partially reversed tactile allodynia produced by reserpine in a dose-dependent manner. However, this drug had no effect on muscle hyperalgesia produced in this model. Treatment with FPS-ZM1 dose-dependently reduced tactile allodynia induced by reserpine. Nonetheless, administration of the RAGE receptor antagonist had no effect on muscle hyperalgesia produced in this model. In contrast, administration of both drug vehicles had no effect on allodynia or hyperalgesia in this model. Moreover, intrathecal injection of TH1020 or FPS-ZM1 (100 nmol, each) had no effect in pain-like behavior in naïve female rats. These data suggest that activation of TLR5 and RAGE receptors have pronociceptive effects in fibromyalgia-like nociception, with a major role of the RAGE receptor. Results suggest that TLR5 and RAGE receptors contribute to the development of reserpine-induced hypersensitivity, specifically tactile allodynia, in this model of functional pain.

Disclosures: L.E. Villanueva-Solis: None. **V. Granados-Soto:** None.

Poster

P391. Pain Models: Pharmacology II

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Topic: D.03. Somatosensation – Pain

Support: NIH Grant NS113991

Title: Inhibition of Nociceptor Endocytosis Attenuates Pain Behavior and Disease Progression in MIA-Induced Osteoarthritis.

Authors: J. TABMAN, ***R. RODRIGUEZ**, A. BHATTACHARJEE;
Univ. at Buffalo - The State Univ. of New York, Buffalo, NY

Abstract: Providing safe and effective pain management for osteoarthritis (OA) has become a critical national problem. Injection of corticosteroids into synovial joints is one of the mainstay therapeutic approaches to treat arthritic pain, however, recent studies have put into question their efficacy and point to potential long-term deleterious effects of steroid therapy. Our previous studies have shown that inhibition of the nociceptor endocytotic adaptor protein complex 2 (AP2) reduces neuronal hyperexcitability suggesting that targeting nociceptor endocytosis provides analgesia. Here we demonstrate that pain behavior in arthritis can be reduced by local pharmacological inhibition of the nociceptor AP2 complex using a AP2 cell-penetrating peptide inhibitor. Monoiodoacetate (MIA) was used to induce knee joint OA in Sprague-Dawley rats, and 4 days later after verification of pain, animals were administered a one-time intra-articular injection of either the AP2 inhibitor peptide or a scrambled peptide control into the arthritic knee. Joint pain was assessed by Dynamic Weight Bearing (DWB) and pain behavior was monitored over the course of 28 days post MIA-induced OA. DWB analyses showed the typical decrease in weight bearing on the arthritic knee injected with the scrambled peptide, however an increase in weight bearing was observed, persisting for up to 24 days in the AP2 peptide injected group, indicating an attenuation of pain behavior. Furthermore, micro-computed tomography and histological analyses were performed on arthritic knees as well as the contralateral healthy knee at end of 28 days to evaluate the effects of scrambled peptide vs the AP2 inhibitor peptide on disease progression. Pathological analyses indicated a reduction of subchondral bone content and cartilage formation in scrambled peptide-injected OA knee joints, but intra-articular AP2 inhibitor peptide injection showed a significant retention of bone content and cartilage, suggesting that blocking nociceptor endocytosis decreases OA pathology. Altogether, this study suggests that nociceptor endocytosis plays an important during OA pain, as well as in the pathophysiological process involved in knee joint degeneration.

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Poster

P391. Pain Models: Pharmacology II

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Topic: D.03. Somatosensation – Pain

Support: NIH Grant AR068989
NIH Grant NS045594

Title: The pronociceptive role of the mineralocorticoid receptor in inflammatory low back pain in mice

Authors: *K. A. QUALLS, W. XIE, J.-T. ZHANG, J. A. STRONG, S. DAVIDSON, J.-M. ZHANG;
Anesthesiol., Univ. of Cincinnati, Cincinnati, OH

Abstract: Inflammatory low back pain is a common and costly condition in the U.S. that is often treated using corticosteroid injections. These treatments can activate the glucocorticoid receptor (GR), and the mineralocorticoid receptor (MR) with similar potency. While steroids are given to bind the GR to relieve inflammation at the dorsal root ganglion (DRG), the MR has been shown to play a pronociceptive role at the DRG in rat models. **The objective of this work is to characterize the impact of conditional MR knockout in mouse sensory neurons on pain behaviors and neuronal excitability during a model of inflammatory low back pain.** In this study, we first characterized and compared expression profiles of the steroid receptors in mouse and human DRGs using RNAscope to perform in-situ hybridization. We then examined the pronociceptive role of the MR in inflammatory back pain in MR KO mice by crossing MR fl/fl mice with Pirt Cre mice, or by hindpaw injection of AAV9 hSyn-eGFP-Cre. MR fl/fl mice served as controls, and all experiments included both sexes and tested for sex differences before combining results. The local inflammation of the DRG (LID) model was implemented to induce long-lasting inflammatory low back pain. Intracellular sharp-electrode recording was done on whole DRG preparations. RNAscope of human and mouse DRG confirmed that both species exhibit widespread expression of GR and MR mRNAs, lending support for using mice as a model organism, and underlining the importance of these receptors at the DRG. During the LID model, MR KO mice showed a reduction in spontaneous and reflexive mechanical pain measures. Electrophysiology analysis of MR KO mice during this pain model revealed divergent effects on A and C cells. MR KO A cells were less likely to fire multiple action potentials at a suprathreshold stimulus, indicating lower excitability, whereas MR KO C cells had a lower rheobase, indicating higher excitability. We can conclude from these data that MR KO in mouse sensory neurons is therapeutic for mechanical pain behaviors during a model of inflammatory

low back pain. MR KO has opposing effects on neuronal excitability in A and C fibers, suggesting that MR plays a distinct role in these different cell types. This work used a targeted genetic approach to confirm that sensory neuronal MR can exacerbate inflammatory pain in a rodent model and supports that MR could have different mechanisms of action in A and C fibers.

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Poster

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Topic: D.03. Somatosensation – Pain

Support: VA I01RX001475
NIH R01NS094438

Title: Autonomic regulation of nociceptive and immunologic changes in a model of complex regional pain syndrome

Authors: *P. SAHBAIE[1,2], W.-W. LI[1,2], T.-Z. GUO[2,3], X.-Y. SHI[1,2], W. S. KINGERY[3], J. CLARK[1,2];
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Abstract: Chronic pain frequently develops after limb injuries, and its pathogenesis is poorly understood. We explored the hypothesis that the autonomic nervous system regulates adaptive immune system activation and nociceptive sensitization in a mouse model of chronic post-traumatic pain with features of complex regional pain syndrome (CRPS). In studies sympathetic signaling was reduced using 6- hydroxydopamine (6-OHDA) or lofexidine, while parasympathetic signaling was augmented by nicotine administration. Hindpaw allodynia, unweighting, skin temperature, and edema were measured at 3 and 7 weeks after fracture. Hypertrophy of regional lymph nodes and IgM deposition in the skin of injured limbs were followed as indices of adaptive immune system activation. Passive transfer of serum from fracture mice to recipient B cell deficient (muMT) mice was used to assess the formation of pain-related autoantibodies. We observed that 6-OHDA or lofexidine reduced fracture-induced hindpaw nociceptive sensitization and unweighting. Nicotine had similar effects. These treatments also prevented IgM deposition, hypertrophy of popliteal lymph nodes, and the development of pronociceptive serum transfer effects. We conclude that inhibiting sympathetic or augmenting parasympathetic signaling inhibits pro-nociceptive immunological changes

accompanying limb fracture. These translational results support the use of similar approaches in trials potentially alleviating persistent post-traumatic pain and, possibly, CRPS.

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Poster

P391. Pain Models: Pharmacology II

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Program #/Poster #: P391.07

Topic: D.03. Somatosensation – Pain

Title: Spontaneous activity in long-term organotypic cultures of the adult human spinal cord

Authors: *V. JEEVAKUMAR, T. INDERSMITTEN, T. COTTA, T. WONG, Y. MIRON, N. ABI-GERGES, A. GHETTI;
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Abstract: Neurons of the spinal cord (SC) form a pattern generator of spontaneous rhythmic bursting activity and oscillations. SC research typically relies on studies conducted with CNS tissue derived from animal models. However, CNS clinical attrition is in part a consequence of the inability of these animal models to generate data predictive of human responses. The ideal test system would utilize human primary tissue, afford reasonable throughput and be highly correlated with clinical outcomes. With our well-established human tissue recovery model from organ donors, we are in an ideal position to perform assays directly on human CNS tissue. To this end, we developed an organotypic slice culture system of adult hSC slices. SCs obtained from organ donors were transported to our laboratory in a protective solution, sliced into 400 µm sections on a vibratome, and cultured in a medium to support neuronal viability up to 27 days in-vitro. We performed Ca^{2+} imaging on the slices to quantify spontaneous activity (SA), focusing on the dorsal horn which receives synaptic inputs from nociceptors in the dorsal root ganglia. Slices were loaded with Fluo-8 AM and imaged on an Olympus IX71 inverted scope at 2 Hz for 10 min of vehicle baseline, followed by 5 min of drug perfusion, and 10 min of acquisition, repeated for each drug / concentration. Neurons were identified as those responding to the application of 1 µM TTX. The persistence of SA was observed on days-in-vitro 20 and 27. Bath application of the AMPAR antagonist CNQX (10 µM) reduced SA, which was subsequently abolished by co-application of the NMDAR antagonist APV (100 µM). Next, we tested Carbamazepine (Tegretol), a Na^{+} channel blocker. Although Carbamazepine is used for the treatment of epilepsy, peripheral neuropathy, and trigeminal neuralgia, it can cause CNS side effects, like dizziness, nausea, mood swings and depression. Therefore, we tested the effects of Carbamazepine on SA at therapeutic exposure (3 µM) and 3- and 10-fold above the therapeutic concentration. SA in the hSC slices was reduced by Carbamazepine in a dose-dependent manner

with an IC_{50} value of 9.2 µM. TTX abolished any remaining SA. Our data shows for the first time that: 1. SA can be reliably measured in adult human CNS tissue up to 4 weeks *in vitro*, which will allow long-term studies of synchronicity, network dynamics and perturbations in the diseased CNS state; 2. Organotypic hSC slice cultures can provide a valuable translational tool for the evaluation of the efficacy and safety of novel CNS drug candidates.

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Poster

P391. Pain Models: Pharmacology II

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Topic: D.03. Somatosensation – Pain

Support: AHA Grant 19AIREA34470007

Title: Modulation of ASIC1b/3 by endogenous molecular zinc

Authors: Q. JIANG, M. WILLIAM, X. ZHA, ***X. CHU**;
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Abstract: Acid-sensing ion channels (ASICs) are proton-gated ion channels and highly expressed in nervous system. Two subunits, ASIC1b and 3, are highly expressed in peripheral sensory neurons. They are sensitive to endogenous biomolecule zinc at micromolar concentrations and plays a critical role in physiological and pathophysiological conditions, especially in pain sensation. Our previous studies have shown that both homomeric ASIC1b and ASIC3 are not sensitive to zinc by co-application. However, pretreatment with zinc dose-dependently inhibits homomeric ASIC1b currents with an IC_{50} of 26 µM and cysteine 149 in the extracellular domain is responsible for this inhibition. While, pretreatment with zinc also inhibits homomeric ASIC3 currents with an IC_{50} of 61 µM in a sharp range. Here, we found that ASIC1b and ASIC3 were co-expressed in CHO cells. Further, the currents recorded from heterologous expressed ASIC1b/3 channels by whole-cell patch-clamp, were modulated by zinc. Co-application of zinc did not affect both peak amplitude and sustained component of heteromeric ASIC1b/3 currents. While pretreatment with zinc induced a significant inhibition of peak amplitude and potentiated the sustained component of heteromeric ASIC1b/3 channels when zinc concentrations were over 300 µM. These findings suggest that endogenously biomolecule zinc is a modulator of heteromeric ASIC1b/3 channels.

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Poster

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Topic: D.03. Somatosensation – Pain

Support: NIH Grant F31 DK12148401
NIH Grant R01 DK115478

Title: Cgrp signaling in the left versus right central amygdala has differential effects on bladder pain modulation in female mice

Authors: *H. N. ALLEN[1,2,3,4,5], B. J. KOLBER[4,5];
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Abstract: The central amygdala (CeA) is a bilateral, mid-brain limbic region that is involved in the processing of pain and emotion. Specifically, the CeA is involved in the processing and modulation of urologic chronic pelvic pain syndromes. Interestingly, the left and right CeA are functionally lateralized and manipulation of left versus right CeA cells has opposing effects on bladder pain. However, the specific mechanisms driving these divergent functions are unknown. Nociceptive signals from the bladder are relayed to the CeA via projection neurons from the parabrachial nucleus (PBN) that express high levels of calcitonin gene-related peptide (CGRP). The aim of this study was to investigate the influence of parabrachial (PBN) CGRP signaling in the left and right CeA on the lateralized modulation of bladder pain. Both optogenetic and pharmacological manipulation of left and right PBN-to-CeA CGRP terminals resulted in opposing effects on bladder pain-like behaviors. Optogenetic activation of CGRP terminals in the left CeA decreased bladder pain-like behaviors while activation of CGRP terminals in the right CeA increased bladder pain-like behaviors. Similarly, pharmacological activation of CGRP receptor cells via CGRP agonist decreased bladder pain when infused in the left CeA but increased bladder pain when infused in the right CeA. Both optogenetic inhibition of CGRP terminals from the PBN in the left CeA and pharmacological inhibition of CGRP receptor cells in the left CeA increased bladder pain-like responses, suggesting that there is ongoing, anti-hyperalgesic CGRP signaling in the left CeA. In a similar but contrasting manner, both optogenetic inhibition of right PBN-to-CeA CGRP terminals and pharmacological inhibition of right CeA CGRP receptor cells decreased bladder pain-like responses, indicating that CGRP signaling in the right CeA is pro-nociceptive. Effects of optogenetic activation of CGRP were able to be blocked by co-infusion with a CGRP antagonist but not a cocktail of AMPA and NMDA receptor antagonists. Similarly, CGRP knock out animals showed no effects of optogenetic manipulation of PBN-to-CeA CGRP terminals, suggesting that CGRP signaling is the driving force of CeA lateralization in the context of bladder pain.

Disclosures: H.N. Allen: None. **B.J. Kolber:** None.

Poster

P391. Pain Models: Pharmacology II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P391.10

Topic: D.03. Somatosensation – Pain

Title: Identification of fungal secondary metabolites with anti-nociceptive activity using a zebrafish model

Authors: *J. A. STROTHER, S. K. ESTES, M. DEADMOND, E. M. MARSHALL, S. LOESGEN;
Univ. of Florida, Saint Augustine, FL

Abstract: Chronic pain is a leading cause of disability and disease and there is an urgent need to develop new therapeutic approaches. Natural products, or compounds isolated from biological sources, have been a reliable source for drug leads and account for a large fraction of clinically approved drugs. However, identifying novel compounds with novel pain-relieving, or anti-nociceptive, activity has been hindered by available testing methods and the difficulty of determining the mechanistic basis of active compounds. To address these challenges, we developed a novel zebrafish-based behavioral assay for anti-nociceptive activity and then surveyed a library of fungal secondary metabolites. We identified several fungal metabolites with previously undescribed anti-nociceptive activity. To determine the mode of action of these compounds, we then used *in vivo* cellular-resolution, whole-brain calcium imaging to locate the neuronal populations affected by active compounds. These results suggest that fungal secondary metabolites are a promising source for anti-nociceptive compounds, and indicate that whole-brain calcium imaging mapping is a valuable tool for understanding the mode of action of active compounds.

Disclosures: J.A. Strother: None. **S.K. Estes:** None. **M. Deadmond:** None. **E.M. Marshall:** None. **S. Loesgen:** None.

Poster

P405. Pain Imaging and Perception IV

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P405.01

Topic: D.03. Somatosensation – Pain

Support: Shirley Ryan AbilityLab - Translational Grant for Pediatric Rehabilitation Research

Title: Brain networks underlying disability in adolescents with chronic pain

Authors: *R. JABAKHANJI[1,2], M. N. BALIKI[4,3,2], D. AMSTUTZ[4,3], G. REVIVO[4,3];
[1]Physiol., [2]Ctr. for Translational Pain Res., [3]Physical Med. and Rihabilitation, Northwestern Univ. Feinberg Sch. of Med., Chicago, IL; [4]Shirley Ryan AbilityLab, Chicago, IL

Abstract: Chronic pain is a serious problem within the pediatric population and is multi-factorial in nature. Here we investigate the behavioral and brain properties associated with various disability measures in adolescents with chronic pain. Nine adolescents with chronic pain (Females, 14-17yo) were recruited from the pain rehabilitation program at the Shirley Ryan Ability Lab. All participants completed the Bath Adolescents Pain Questionnaire (BAPQ), the Tampa Scale for Kinesiophobia (TSK), the Canadian Occupational Performance Measure (COPM), a 6 Minute Walk Test (6MWT), and rated their pain (0-10). Parents completed the Bath Adolescent Pain Parental Impact Questionnaire (BAP-PIQ). We also collected one anatomical (T1-weighted), and two resting state MRI scans per subject.Unique disability measures were identified using PCA analysis. All disability measures loaded on 3 orthogonal factors mainly 1) functional disability (FD) that included BAPQ - Daily Function, TSK, and 6MWT, 2) Occupational disability (OD) that included BAPQ-Development and COPM and 3) emotional disability that included BAPQ-Emotional Functioning. Pain intensity was only associated to FD ($r=-0.59$, $p=0.096$), while pain duration was correlated to ED ($r = 0.56$, $p=0.12$). Finally, OD was associated with the parent catastrophizing subscale ($r =0.53$, $p=0.13$). Resting state data was analyzed using standard methods. Following image preprocessing and registration, we utilized a standard parcellation schema to determine the correlation between defined brain networks. Association between network connectivity and disability measures were then identified using Pearson correlation ($p<0.05$). FD was mainly associated with decreased cingulo-opercular (CO) - memory retrieval (MR) networks connectivity as well as decreased and Dorsal attention (DA)- salience (Sal) networks connectivity. In contrast, OD was related to increased DA - somatosensory (SS) networks connectivity, and negatively correlated with the frontoparietal (FP) - subcortical networks connectivity. Finally, ES was related to decreased DMN – subcortical network connectivity and increased DMN connectivity to somamatosensory regions.Overall, our results show that pain related disability in adolescents exhibit unique behavioral and brain mechanisms that may aid clinics in optimizing pain rehabilitation protocols.

Disclosures: R. Jabakhanji: None. **M.N. Baliki:** None. **D. Amstutz:** None. **G. Revivo:** None.

Poster

P405. Pain Imaging and Perception IV

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P405.02

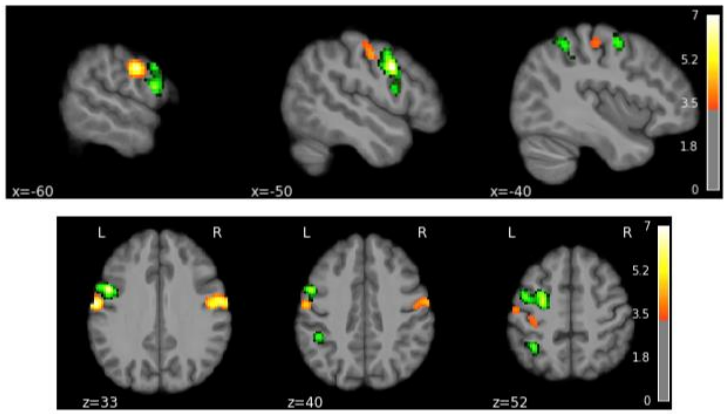
Topic: D.03. Somatosensation – Pain

Support: MRC Career Development Award MR/T010614/1
Wellcome Trust 097490

Title: Learning the temporal statistics of pain states: computational mechanisms and neural correlates

Authors: *F. MANCINI[1], S. ZHANG[2], B. SEYMOUR[3];
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Abstract: Pain invariably changes over time, and these temporal fluctuations are riddled with uncertainty about body safety. In theory, statistical regularities of pain through time contain useful information that can be learned, allowing the brain to generate expectations and inform behaviour. To investigate this, we exposed healthy participants to long probabilistic sequences of low and high pain stimuli (electrical pain to the left hand), containing sudden changes in background statistics (frequencies). We demonstrate that humans can learn to extract these regularities, and explicitly predict the likelihood of forthcoming pain intensities in a manner consistent with optimal Bayesian models. We studied brain activity using fMRI whilst subjects performed the task, which allowed us to dissect the underlying neural correlates of these statistical inferences from its statistical surprise. We found that the inferred frequency (posterior probability) of high intensity pain correlated with activity in bilateral sensorimotor cortex and caudate, right secondary somatosensory cortex and nucleus accumbens. This activity therefore tracks the background frequency of high pain states, over and above the encoding of stimulus intensity. An intrinsic part of this hierarchical Bayesian model is the way that unexpected changes in frequency lead to re-learning, and this is reflected by a type of prediction error (KL divergence between consecutive posterior distributions). This was associated with brain responses in the premotor cortex (green clusters in fig), adjacent to the regions inferring the frequency of high pain states (yellow clusters in fig). In conclusion, we show that humans can infer the background temporal statistics of pain in a Bayesian manner, and that distinct sensorimotor and premotor subregions dynamically encode temporal inferences and their prediction error.



Overlaying Bayesian prediction error with temporal inference of high pain (posterior mean).
FDR corrected p<0.001, colorbar shows Z scores, thresholded at Z>3.3.

Disclosures: F. Mancini: None. **S. Zhang:** None. **B. Seymour:** None.

Poster

P405. Pain Imaging and Perception IV

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P405.03

Topic: D.03. Somatosensation – Pain

Support: NCCIH K99/R00-AT008238
NCCIH R01-AT009693
NCCIH R21-AT010352

Title: Neurofunctional connections supporting mindfulness-based pain relief

Authors: *G. RIEGNER[1], G. POSEY[2], V. OLIVA[1], L. KHATIB[1], J. BAUMGARTNER[1], R. KRAFT[2], Y. JUNG[3], F. ZEIDAN[1];
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[3]Radiology, Univ. of California, Davis, Davis, CA

Abstract: Mindfulness meditation reduces pain through multiple brain mechanisms. Yet, those involved explicitly during meditation and a painful stimulus are poorly characterized. The proposed pre-registered study (NCT03414138) employed multiple neuroimaging analytical methods to determine a) if mindfulness reduces the *neurologic pain signature* (NPS), a multivariate pattern of voxel weights predictive of pain intensity and, b) the neurofunctional connections supporting mindfulness-based pain relief during a noxious stimulus.

After a baseline testing session, 40 pain-free participants (mean age = 30 years, 20 females) were randomized to four, 20-minute sessions of mindfulness training or book listening. Following completion of the interventions, fMRI scans were acquired during noxious heat stimulation of the right calf (ten, 12-second 49°C trials; Medoc Inc). All subjects rested pre-manipulation and then meditated (mindfulness group) or continued to rest (control group). After each scan, visual analog scale (VAS) ratings of pain intensity (0 = "no pain" to 10 = "worst pain imaginable") were collected. A 2 (group) X 2 (pre- vs post-manipulation) RM ANOVA determined if mindfulness significantly reduced pain. A paralleling whole-brain RM ANOVA was conducted (FSL 6.0.4). For each fMRI scan, a vector of regression coefficients corresponding to voxel activations was compared to the NPS by the dot-product, returning a scalar value representing pattern similarity. Additionally, within-group ventromedial PFC (vmPFC) (derived from a large meta-analysis) to whole-brain functional connectivity was performed. Cluster-level inference was defined by a threshold of $p < .001$ and family-wise error rate of $p < .05$.
The interaction on VAS pain ratings, $p < .001$, $\eta p^2 = .41$, was driven by the reduction in pain during mindfulness (-32%) when compared to controls (+20%). The NPS interaction, $p = .002$, $\eta p^2 = .23$, was associated with a response reduction in the meditation group ($p = .006$) but not the controls ($p = .10$). The whole-brain GLM demonstrated that mindfulness-based pain relief was associated with greater vmPFC deactivation. Functional connectivity analyses revealed that weaker vmPFC connectivity with the contralateral anterior insula and fronto-parietal operculum predicted greater analgesia.
These preliminary findings demonstrate that mindfulness reliably reduces direct behavioral and neural mechanisms of pain. We also reveal that mindfulness-induced pain relief is associated with greater decoupling between the vmPFC, a brain mechanism critical for self-related appraisals of the sensory environment, and brain regions supporting sensory pain discrimination.

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Poster

P405. Pain Imaging and Perception IV

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P405.04

Topic: D.03. Somatosensation – Pain

Support: DOD grant W81XWH-15-1-0603
NIH grant P50 DA044121
NIH grant R01AR074274-01A1

Title: Structural brain connectivity predetermines early acute pain in mild traumatic brain injury

Authors: *P. BRANCO[1], N. BOSAK[2], O. CONG[1], J. BIELEFELD[1], Y. GRANOVSKY[2], D. YARNITSKY[2], A. APKARIAN[1];
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Abstract: Acute pain is one of the most debilitating symptoms of mild traumatic brain injury (mTBI). It can last for days-to-weeks, and, for many patients, it can persist for years; and its magnitude is a strong predictor of chronic pain post mTBI. There is a poor relationship between pain reports, tissue injury, and imaging findings of the injured tissue. Here we test the hypothesis that mTBI acute pain (within 48 hours of car accident) is in part predicted by brain structural and resting state connectivity. Resting state fMRI (rsfMRI), anatomical (T1) and white matter (DTI) brain scans were collected in 155 mTBI patients immediately after a car accident (within 48 hours). All participants also underwent pain psychophysical testing, and clinical and demographic measures were also collected. We used a robust machine-learning pipeline to map acute pain to DTI and rsfMRI connectivity properties. Brain predictors of acute pain were then related to clinical and pain related measures. We show that white-matter brain properties - but not functional properties - explain a quarter of the variance in pain ratings after injury; this finding replicated in two additional independent samples of 38 and 13 patients with and without whiplash-like symptoms, respectively. These findings were not dependent on injury-related clinical or demographic characteristics. Instead, white matter properties mapped to conditioned pain modulation and pain sensitivity scores. Together, these parameters could explain a third of the pain variability irrespective of tissue damage. White matter connectivity showed strong within-subject reliability because it did not change up to a year after the injury; and connectivity metrics collected at baseline, as well as at six months, were able to predict subjects' pain up to six months after the initial injury. The study is the first evidence that brain structural connectivity properties can determine a large portion of the variance of acute pain for mTBI. As these properties were invariant over months from the injury and could still be related to mTBI pain, we conclude that they are preexisting properties. We speculate that resting state fMRI could not be related to the acute mTBI pain as it may be perturbed by multiple negative emotional states during acute mTBI.

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Poster

P405. Pain Imaging and Perception IV

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Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P405.05

Topic: D.03. Somatosensation – Pain

Support: NIH/NIDA P50 DA044121-01A1
Craig H. Neilsen

Title: disability is primary mediated by pain intensity and avoidance in patients with chronic back pain.

Authors: *M. N. BALIKI[1,3], B. WU[4], K. WAKAIZUMI[1], R. JABAKHANJI[2], L. HUANG[2], A. V. APKARIAN[2];
[1]Physical Med. and Rehabil., [2]Physiol., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL; [3]Shirley Ryan Ability Lab., Chicago, IL; [4]Pain Med., Wenzhou Med. University,, Wenzhou, China

Abstract: Patients with chronic back pain (CBP) and disability represent a formidable challenge to scientists and health professionals. A better understanding of the behavioral and neural factors affecting disability can provide a conceptual framework for the evaluation and treatment of CBP patients. In this study we determine the demographic, pain, and psychological properties associated with disability and then identify the unique brain networks mediating their effects using resting state fMRI (rs-fMRI).
We recruited 2 groups of CBP patients for this study. Group 1 (n=143) was used to investigate the behavioral correlates of pain disability, while group 2 (n= 130) was used for validation . Disability was assessed using the Oswestery disability index (ODI). In addition, patients completed various questionnaires including numerical pain rating (NRS), pain duration, exercise and smoking frequency, pain anxiety symptom scale (PASS), pain catastrophizing scale (PCS), Becks depression index (BDI), Positive and negative affect schedule (PANAS). Finally, rs-fMRI and anatomical images were collected for all patients in Group2.
The relationship between ODI and behavior was determined using multiple linear regression with cross-validation in group 1. Only NRS and PASS-avoidance showed significant regression across all folds ($R^2 = 0.48$, $p<0.01$). This relationship was validated in group 2 ($R^2=0.44$, $p<0.001$). rs-FMRI dawas analyzed using standard methods. Following image preprocessing, we utilized a standard parcellation schema to determine the correlation between 260 ROIs across 12 defined brain networks. Functional connectivity associated with ODI was determined using robust regression ($p<0.05$, FDR corrected). ODI was associated with 72 pair-wise connections mainly localized to somatosensory, default-mode and salience networks. In order to determine whether this connectivity patter mediated the effect of pain and PASS-avoidance on disability, we first used a PCA analysis to decompose the 72 “betas” into 20 orthogonal components that accounted to 80% of the variance. The components were submitted to mediation analysis. Only component 1 significantly mediated the effect of pain intensity (a, b, ab paths $p<0.001$) as well as PASS-avoidance (a, b, ab paths $p<0.05$). Finally we validated our mediation results in 20 patients with high-impact pain.
Overall our results show that disability in chronic pain patients is primary mediated by pain intensity and avoidance. Therefore therapeutic approaches specific targeting these clinical properties may be successful in managing disability in chronic pain patients.

Disclosures: M.N. Baliki: None. **B. Wu:** None. **K. Wakaizumi:** None. **R. Jabakhanji:** None. **L. Huang:** None. **A.V. Apkarian:** None.

Poster

P405. Pain Imaging and Perception IV

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P405.06

Topic: D.03. Somatosensation – Pain

Support: P50 DA044121
R01AR074274-01A1

Title: Constituent dimensions of chronic pain domains and their relationship to psychology and brain connectivity.

Authors: *C. BONIN PINTO[1,2], J. BIELEFELD[2,1], J. BARROSO[2,1], B. YIP[3], L. HUANG[1,2], M. N. BALIKI[4,2], T. SCHNITZER[3,2], V. APKARIAN[1,2];
[1]Physiol. Dept., [2]Ctr. for Translational Pain Res., [3]Dept. of Physical Med. and Rehabil., Northwestern Univ., Chicago, IL; [4]Rehabil. Inst. of Chicago, Chicago, IL

Abstract: Chronic pain is a multidimensional pathological state. Evidence suggests that specific brain properties and patients' psychological and performance ability traits are modified in chronic pain patients. However, the relationship between these alterations and pain dimensions remains poorly understood.In this cross-sectional analysis, we evaluated multiple dimensions of chronic pain and its relationship with brain connectivity and measures of personality, social interaction, psychological and ability traits (bio-psycho-social traits). By assessing a broad battery of pain-related outcomes questionnaires of 107 chronic low back pain patients we show that chronic pain is characterized by three distinct domains - pain magnitude, pain affect & disability and pain quality. Next, we modeled the relationship between each of these domains and the bio-psycho-social traits. Pain magnitude (OOS $r^2 = 0.33$) and pain affect & disability (OOS $r^2 = 0.79$) were significantly associated with different patterns of patients' bio-psycho-social traits. Pain quality did not relate significantly to these variables. Interestingly, while mindfulness and awareness were major contributors of pain magnitude, social relationship dysfunction seem to be the major contributor for higher pain affect & disability scores.Lastly, we investigated whether specific patterns of brain activity were related to each of the chronic pain domains. Pain magnitude was associated with the sensorimotor and the cingulo-opercular task control networks (OOS $r^2=0.41$). Pain affect & disability related to the functional connectivity within the frontoparietal task control and default mode networks (OOS $r^2 = 0.35$). Finally, pain quality integrated the sensorimotor, auditory, and cingulo-opercular task control networks (OOS $r^2 = 0.43$). By using Neurosynth to provide a meta-analytic summary of terms related to these brain networks, we show that pain magnitude relates mostly to motor and sensory terms, pain affect & disability relates to brain regions associated with memory terms, and the pain quality domain to both memory and motor and sensory terms. Finally, a mediation analysis showed that

the relationship between the bio-psycho-social traits and the pain domains is only partially mediated by the brain functional connectivity networks.Our results show the high-level dimensionality of chronic pain and how distinct facets of the pain experience have different bio-psycho-social correlates and can be determined by unique brain network signatures.

Disclosures: C. Bonin Pinto: None. **J. Bielefeld:** None. **J. Barroso:** None. **B. Yip:** None. **L. Huang:** None. **M.N. Baliki:** None. **T. Schnitzer:** None. **V. Apkarian:** None.

Poster

P405. Pain Imaging and Perception IV

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P405.07

Topic: D.03. Somatosensation – Pain

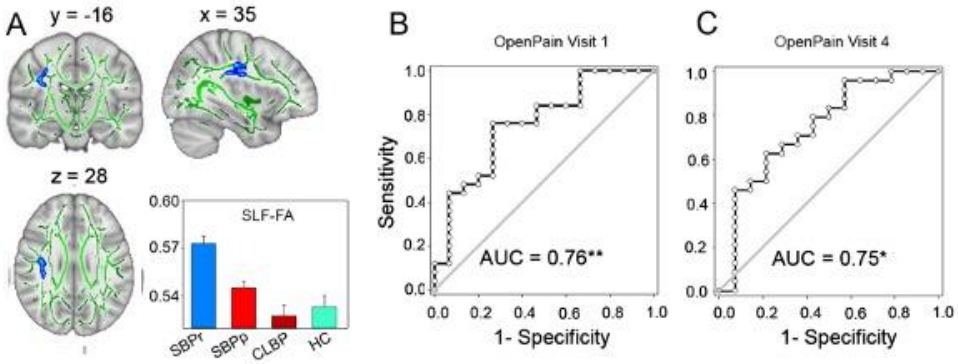
Support: NIDA K08DA037525

Title: A Novel White Matter Predictor of Resilience to Pain Chronification

Authors: N. LEE[1], Z. ZHANG[2], K. MURRAY[3], ***P. GEHA**[4];
[1]Dept. of Psychiatry in Univ. of Rochester, Rochester, NY; [2]Univ. of North Carolina at Chapel Hill, North Carolina, NC; [3]Universiyt of Rochester, Rochester, NY; [4]Univ. of Rochester, NY, NY

Abstract: Early identification of the likelihood of pain "chronification" is a pre-requisite step for the efficient implementation of preventive treatment. However, validated quantitative prognostic biomarkers that can predict disease trajectory of acute or chronic pain have yet to be identified. Here we identify a brain white matter biomarker of resilience to chronic pain in our data set and validate it on 2 data sets originating at a different site. 27 patients suffering from new onset sub-acute low-back pain (duration 6-12 weeks) were studied at baseline and at 1-year follow-up. 15 patients recovered (SBPr) at follow-up as their pain dropped by > 30% and 12 patients persisted in having pain (SBPp). All patients underwent structural brain imaging to obtain an anatomical and diffusion weighted images (DWI) both at baseline and at follow-up. We compared white matter properties (fractional anisotropy, FA) between SBPr and SBPp patients using tract based spatial statistics (Smith *et al.*, 2006) and using a novel population based structural connectome (SC) (Zhang *et al.*, 2018). T1 and DWI data was also obtained from www.openpain.org from 15 SBPr and 25 SBPr patients both at baseline and at one year follow-up. This data was used to validate our results. Unpaired t-test comparison between SBPr and SBPp patients at baseline revealed increased FA in the right superior longitudinal fasciculus (RSLF) of SBPr patients ($p < 0.05$, whole brain corrected against 5000 permutations) (**Figure 1A**). FA values extracted from the RSLF in the validation data collected at a different site accurately classified SBPr and SBPp patients with an area under the receiver operating

characteristic curve of > 0.75 both at baseline and at follow-up (**Figure 1B-C**). Preliminary study of the SC revealed decreased white matter fiber count ($p < 0.05$, corrected) in SBPr patients between brain areas falling within the right parietal lobe. We conclude therefore that the local properties of the RSLF and its structural connectivity are potential biomarkers for resilience to pain chronification, a result validated across sites.



Disclosures: N. Lee: None. **Z. Zhang:** None. **K. Murray:** None. **P. Geha:** None.

Poster

P405. Pain Imaging and Perception IV

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P405.08

Topic: D.03. Somatosensation – Pain

Support: NIH/NIA K01AG048259
NIH/NIA P30AG028740-14S1
NSF Cooperative Agreement No. DMR-1644779
NSF Cooperative Agreement No. DMR-1157490,
State of Florida

Title: Sex-related differences in brain morphology in chronic musculoskeletal pain in older adults

Authors: *P. VALDES-HERNANDEZ[1], S. MONTESINO-GOICOLEA[1], A. JOHNSON[1], C. LAFFITTE-NODARSE[1], J. PERAZA-GOICOLEA[1], R. FILLINGIM[1], Y. CRUZ-ALMEIDA[2]; [2]Inst. on Aging, [1]Univ. of Florida, Gainesville, FL

Abstract: Musculoskeletal pain (**MSK**) affects millions of US adults, especially older populations. The neuroanatomical underpinnings of reported differences in prevalence of MSK between females (**Fs**) and males (**Ms**), as well as in experimental and clinical pain measures remain understudied, especially in older adults. This study aimed to compare brain morphological measures (**BMM**) among sexes. Our sample had 77 individuals 61-94 years with and without MSK (**P and NP groups**) from the *Neuromodulatory Examination of Pain and Mobility Across the Lifespan* (*NEPAL*) study at the University of Florida. Participants completed demographic, psychological, and clinincal/experimental pain assessments (**Vars**). T1-weighted anatomical images were acquired with a 3T scanner. Using FreeSurfer (v.7.1.0), cortical volume, areas (**CA**) and thickness (**CT**) and subcortical volumes were calculated in selected areas of the Destrieux atlas known to be related to pain processing (subcentral, frontal, occipitotemporal, postcentral, insulae, cingulate, hippocampi and basal ganglia). Statistical tests were corrected for multiple comparisons using False Discovery Rate ($p<0.05$). We investigated the extent of BMM sex differences on P and NP (sex-by-pain interaction), controlling for age, race and total intracranial volume. This interaction was significant in CA of the left inferior segment of the circular sulcus of the insula (**ICIns-L**). This solely owed to a greater CA in Ms than Fs (significant simple effect of sex) in P, since no significant difference among sexes (no significant simple effect of sex) were found in NP. The interaction was also significant on CT of the middle-anterior part of the cingulate gyrus and sulcus, solely owing to a smaller CT in Ms in P (i.e., there was a significant simple effect of sex in P, no significant simple effect of sex in NP, and no significant simple effect of pain in Fs, but only in Ms). Post-hoc analysis evaluated the moderation of Vars on the sex differences in BMMs in P. The number of anatomical pain sites (**APSites**) and positive scale of the Positive and Negative Affect Schedule (**PANAS-pos**) significantly moderated the sex difference in CA of ICIns-L. Johnson-Neyman (**JN**) analyses showed that this sex difference was significant for APSites equal 3 or higher, where CA in Ms was significantly greater for lower APSites, while did not significantly change across APSites values in Fs. JN also showed that the sex difference in CA of ICIns-L was significant for PANAS-pos higher than 33, being greater for Ms and smaller for Fs for higher PANAS-pos. We provide initial evidence for sex differences in brain structure in MSK in older adults, which may be related to psychological processes.

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Poster

P474. Visual Cortex: Functional Architecture and Circuits

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P474.01

Topic: D.07. Vision

Title: Poor spatial representation in visual cortex in the absence of visual input

Authors: *C. WILHITE[1], Y. SENZAI[2], M. SCANZIANI[2];
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Abstract: Recent studies using virtual reality environments in mice have demonstrated that neuronal responses in primary visual cortex (V1) are modulated by spatial location[1,2]. It is unknown, however, whether these signals reflect a higher-order spatial code that can be maintained by self-motion or other sensory cues in the absence of visual input. To explore this question, we record V1 units in freely moving mice on a linear track in various levels of ambient luminance, including pitch darkness. Prior to recording, mice were adequately familiarized on the track in both light-on and pitch-dark conditions. Many cells in V1 maintain consistent spatial modulation across a wide range of luminance levels. In pitch darkness, however, overall spatial information in V1 is strongly reduced and the locations of peak firing fields remap across the entire length of the track. Furthermore, units in V1 that are significantly modulated by spatial location in pitch darkness tend to have peak firing fields at the edges of the track, resulting in reduced spatial coverage compared to light-on conditions. These data indicate that even in familiar environments, spatial representation in V1 cannot be maintained by self-motion or other sensory cues in the absence of visual input.
References:
1. Fiser A, Mahringer D, Oyibo HK, Petersen AV, Leinweber M, Keller GB. Experience-dependent spatial expectations in mouse visual cortex. Nat Neurosci. 2016 Dec;19(12):1658-1664.
2. Saleem AB, Diamanti EM, Fournier J, Harris KD, Carandini M. Coherent encoding of subjective spatial position in visual cortex and hippocampus. Nature. 2018 Oct;562(7725):124-127.

Disclosures: C. Wilhite: None. **Y. Senzai:** None. **M. Scanziani:** None.

Poster

P474. Visual Cortex: Functional Architecture and Circuits

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P474.02

Topic: D.07. Vision

Support: Jane Coffin Childs Memorial Fund
Japan Society for the Promotion of Science

Howard Hughes Medical Institute

Title: Direction selectivity is scrambled in visual cortex during saccades

Authors: *S. K. MIURA[1], M. SCANZIANI[2];
[1]Univ. of California San Francisco, San Francisco, CA; [2]Univ. of California, San Francisco, San Francisco, CA

Abstract: Sensory stimuli are often evoked by the animal's own actions. In both vertebrates and invertebrates, nervous systems distinguish these self-generated stimuli from stimuli evoked by changes in the environment. How this distinction is achieved has been the subject of much investigation, especially in the context of saccades. Saccades are rapid eye movements performed by many animals across phyla, and they induce a quick motion of the visual scene on the retina. While behavioral studies indicate that this saccade-induced motion is distinguished from motion that occurs in the environment, the neural mechanisms by which visual systems differentiate the two types of motion are not fully understood. We addressed this issue in mouse primary visual cortex (V1). We report that saccade-induced motion and motion in the environment evoke distinct patterns of activity in V1, even when the resulting visual stimuli on the retina are identical. This is because during saccades, neurons in V1 linearly integrate the visual input with a strong, non-visual input arriving from the pulvinar nucleus of the thalamus. This non-visual input arrives in V1 prior to the initiation of saccades and encodes the direction of the eye movement. Silencing the pulvinar prevented the non-visual input from reaching V1, such that the pattern of activity in V1 was now similar no matter whether the motion was generated by saccades or by changes in the environment. Thus, our work reveals the mechanisms through which the non-visual input ensures differential responses to self-generated motion and external motion in V1.

Disclosures: S.K. Miura: None. **M. Scanziani:** None.

Poster

P474. Visual Cortex: Functional Architecture and Circuits

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Program #/Poster #: P474.03

Topic: D.07. Vision

Support: NIH Grant R01EY025668
Howard Hughes Medical Institute
NIH Grant U01NS108683

Title: Inter- and intra-hemispheric sources of vestibular signals to the primary visual cortex

Authors: *G. BOUVIER[1], A. SANZENI[2], N. BRUNEL[2], M. SCANZIANI[1];
[1]Univ. of California, San Francsico, San Francisco, CA; [2]Neurobio., Duke Univ., Durham, NC

Abstract: Sensations often trigger movements, but one's movements also generates sensory experience. Vestibular organs monitor head movements and contribute to the brain's ability to distinguish between self- and externally-generated sensory stimuli. Signals from the vestibular organs are broadcasted throughout the brain, including primary sensory regions like the primary visual cortex (V1). However, what type of signals relative to head movements reach V1 and what the upstream sources of these signals are, remain poorly understood. Here we show that, in the absence of visual input, V1 neurons encode the direction of head rotation as well as the angular velocity and acceleration in a layer specific manner. We also discovered that the ipsilateral latero-posterior/centro-lateral thalamus (LP/CL) and the contralateral visual cortex are key source of vestibular input to V1. The ipsilateral LP/CL provides the main head movement signal and this signal is biased toward contraversive head movements (i.e. clockwise head movements when recording from left V1). Instead, the contralateral visual cortex provides head-movement signals during ipsiversive head movements. Thus, this study reveals a direction specific recruitment of intra- and interhemispheric sources of vestibular signals to V1.

Disclosures: G. Bouvier: None. **A. Sanzeni:** None. **N. Brunel:** None. **M. Scanziani:** None.

Poster

P474. Visual Cortex: Functional Architecture and Circuits

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P474.04

Topic: D.07. Vision

Title: Functional and anatomical map of a visual cortex for the superior colliculus

Authors: *J. BRENNER[1], R. BELTRAMO[2], M. SCANZIANI[3];
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Abstract: Studies of cortical visual processing in mammals have traditionally focused on the visual pathway that ascends from the retina to primary visual cortex (V1) via the lateral geniculate nucleus of the thalamus, the so-called geniculate pathway. However, visual information can also reach cortex through an alternate "extrageniculate" visual pathway in which retinal projections to the superior colliculus (SC) are relayed through the pulvinar nucleus of the thalamus (PN) before radiating to various regions of the visual cortex. Recent experiments in the mouse (Beltramo 2019) have demonstrated that this extrageniculate pathway is the principal driver of visually evoked activity in one higher-order visual area of the mouse called postrhinal

cortex (POR). However, the extent to which the extrageniculate pathway drives visually evoked activity in other higher visual areas remains uncertain. We used widefield calcium imaging to map the functional impact of the mouse SC on higher visual cortices. Tetrodotoxin injections in SC revealed that, in addition to POR, visual responses in several higher visual areas depend on SC. These include the laterointermediate area (LI), the posterior area, and, partially, the lateromedial area. Blocking synaptic transmission in widefield SC neurons (tagged by the NTSR1-209 line) via the conditional expression of tetanus toxin light chain strongly attenuated visual responses in POR and LI, establishing SC widefield neurons as a main source for SC mediated visual responses in higher visual areas. Using a transsynaptic anterograde tracing approach, we delineated the disynaptic projections from SC through PN to visual cortex and found that the extent of these projection within each higher visual area matched our functional mapping. Finally, we compared the responses for each higher visual area to self versus externally generated motion of visual stimuli. For this experiment, we presented stimuli moving horizontally in the nasotemporal direction such that the speed of the stimulus was either coupled or uncoupled to the running speed of the mouse on a circular treadmill. POR responded preferentially to exogenously generated motion as compared to V1, specifically when the stimuli moved faster than expected from the running speed of the animal. This work thus delineates a map of higher visual areas in mouse visual cortex whose visual responses depend on the SC and identifies the SC neurons that mediate those responses. At least one of these higher visual areas, POR, shows higher selectivity than V1 to external as compared to self- generated visual motion.

Disclosures: J. Brenner: None. **R. Beltramo:** None. **M. Scanziani:** None.

Poster

P474. Visual Cortex: Functional Architecture and Circuits

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Program #/Poster #: P474.05

Topic: D.07. Vision

Support: R01EY028905
R21EY030291

Title: Corticotectal Neurons and Putative Fast Spike Interneurons: Distinct Properties of Two Types of Complex Cells in Layer 5 of Primary Visual Cortex

Authors: *C. SU[1], Y. BERESHPOLOVA[1], R. F. MENDES-PLATT[1], S. E. KIM[1], J.-M. ALONSO[2], H. A. SWADLOW[1];
[1]Dept. of Psychological Sci., Univ. of Connecticut, Storrs, CT; [2]Biol. and Visual Sci., SUNY Optometry, New York, NY

Abstract: The receptive fields (RFs) of most neurons of primary visual cortex can be classified as simple or complex, either using the classical criteria of Hubel and Wiesel (1962) or by linearity of their responses to drifting gratings (F1/F0 ratios) (Skottun et al., 1991). Here, we investigate two types of complex cells found in visual cortical layer 5 of awake rabbits: (1) corticotectal (CTect) neurons, and (2) putative fast-spike inhibitory interneurons (suspected inhibitory interneurons, SINs). The CTect neurons were identified by antidromic responses following electrical stimulation of the superior colliculus, and the SINs by their high-frequency response to electrical stimulation of the thalamus and by short-duration spikes (3+ spikes at >600 Hz). All of the CTect neurons (n = 29) and all of the SINs (n = 16) had complex RFs, as defined by nonlinear responses to a drifting grating (F1/F0 ratios of <1) presented at near-optimal orientation, spatial frequency, temporal frequency and contrast. CTect neurons and SINs were very similar in their (1) response nonlinearity (0.39 ± 0.05 vs. 0.28 ± 0.06, $P = 0.15$, Wilcoxon test for all comparisons), (2) median response latency to stationary visual stimuli (32.2 ms vs. 31.5 ms, $P = 0.83$), (3) evoked responses to optimal drifting gratings (25.06 ± 3.03 spks/s vs. 41.23 ± 9.96 spks/s, $P = 0.21$), (4) spatial tuning properties (peak spatial frequency: 0.31 ± 0.07 cpd vs. 0.43 ± 0.08 cpd, $P = 0.12$.; spatial frequency bandwidth: 0.56 ± 0.10 oct vs. 0.61 ± 0.07 oct, $P = 0.4$), and the contrast generating half-maximum response (C50) (14.82 ± 2.54 % vs. 14.18 ± 2.0 %, $P = 0.57$). However, they were distinct in a number of ways: (1) Whereas all SINs had complex RFs as defined by low F1/F0 ratios, and had highly overlapping ON and OFF subfields, the spatial RFs of CTect neurons were more diverse, with ~50% showing some significant separation of ON and OFF subregions. (2) CTect neurons had much lower spontaneous activity than L5 SINs (5.86 ± 0.62 spks/s vs. 14.98 ± 2.36 spks/s, $P < 0.001$). (3) CTect neurons showed higher response reliability (lower Fano factor) than L5 SINs (1.00 ± 0.14 vs. 1.76 ± 0.27, $P = 0.005$). (4) Most CTect neurons were selective for stimulus orientation and many of these were directionally selective. However, L5 SINs were broadly tuned to stimulus orientation and direction (orientation selective index: 0.55 ± 0.04 vs. 0.28 ± 0.05, $P = 0.001$; direction selective index: 0.43 ± 0.06 vs. 0.12 ± 0.03, $P = 0.002$). We conclude that layer 5 complex cells are heterogeneous in their RF properties, and that this heterogeneity is related to morphologically and physiologically distinct cell types found in this layer.

Disclosures: C. Su: None. **Y. Bereshpolova:** None. **R.F. Mendes-Platt:** None. **S.E. Kim:** None. **J. Alonso:** None. **H.A. Swadlow:** None.

Poster

P474. Visual Cortex: Functional Architecture and Circuits

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Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P474.06

Topic: D.07. Vision

Support: R01EY028905

R21EY030291

Title: Pronounced differences in retinotopic scatter between putative inhibitory and excitatory neurons in rabbit visual cortex

Authors: *Y. BERESHPOLOVA[1], C. SU[1], R. F. MENDES-PLATT[1], S. E. KIM[1], J.-M. ALONSO[2], H. A. SWADLOW[1];
[1]Dept. of Psychological Sci., Univ. of Connecticut, Storrs, CT; [2]Biol. and Visual Sci., SUNY Optometry, New York, NY

Abstract: Neighboring neurons in primary visual cortex respond to similar stimulus positions in the retina and their retinotopic scatter is very limited in cats and primates. Here we show that, in rabbits, the retinotopic scatter is strongly dependent of cell type. It is 2-4 times larger for layer 4 (L4) simple cells and layer 5 (L5) corticotectal cells (CTect) than for fast-spike suspected inhibitory interneurons (SINs) in both layers. To estimate the retinotopic scatter, we simultaneously measured the receptive fields (RF) of single cortical cell and neighboring multiunit activity (MUA) using reverse correlation from sparse noise stimuli (light and dark squares of 1-2 deg2). Then, we compared the RF positions of MUA and neighboring single neuron by calculating the distance between their RF centers. We classified the RFs as either simple or complex based on the linearity of spatial summation to near-optimal drifting grating stimuli (F1/F0 ratios, complex: <1.0, simple: > 1.0). In L4, the complex cells studied were SINs, identified by high-frequency burst of spikes (> 600 Hz) elicited by thalamic electrical stimulation and very short duration spikes (Swadlow, 2003). In L5, we also studied SINs, as well as antidromically identified Ctect neurons (all of which had F1/F0 ratios of <1, see accompanying poster by C.Su et al). The ON and OFF subregions of the MUA RF were very closely aligned in the two layers (ON-OFF difference in RF center position: 0.95 ± 0.1 deg, n= 29 in L4 vs 0.86 ± 0.08 deg, n = 35 in L5, P = 0.243, Wilcoxon test for all comparisons). The RF centers of SINs were also closely aligned with the center of the MUA RF in both layers, with the alignment being slightly more precise in L4 (MUA-SIN difference in center position: 0.72 ± 0.11 deg, n = 11 in L4 vs 1.02 ± 0.11 deg, n = 17 in L5, P = 0.034). When compared with the SINs, the RF centers of L5 Ctect cells and L4 simple cells were 2-4 times more scattered and frequently found at the edges of the MUA RF (difference in center position: 1.77 ± 0.24 deg, n = 18 L5 Ctect vs. 2.7 ± 0.43 deg, n= 18 L4 Simple, P = 0.243). The differences in RF scatter between SINs and non-SIN neurons were very pronounced and highly significant (P < 0.001 for L4, P = 0.005 for L5). Since the average RF size of a geniculate input at the same eccentricity is 3.9 degrees (n = 57), the average retinotopic scatter of non-SIN neurons spanned from 0.4 (L5) to 1 (L4) geniculate RF centers. We conclude that non-SIN neurons display greater retinotopic scatter than SIN neurons consistently with their more limited thalamocortical convergence (Bereshpolova et al., 2020). The pronounced retinotopic scatter of non-SIN neurons may explain the absence of orientation columns in rabbit visual cortex.

Disclosures: Y. Bereshpolova: None. **C. Su:** None. **R.F. Mendes-Platt:** None. **S.E. Kim:** None. **J. Alonso:** None. **H.A. Swadlow:** None.

Poster

P474. Visual Cortex: Functional Architecture and Circuits

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Program #/Poster #: P474.07

Topic: D.07. Vision

Support: NIMH Grant MH063912
NEI Grant EY022577

Title: Functional interlaminar connectivity in mouse primary visual cortex

Authors: *M. ROSSA, E. M. CALLAWAY;
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Abstract: One of the defining characteristics of the cerebral cortex is its organization into 6 layers, each distinguishable by a unique composition of cell types and together representing a fundamental component of sensory information-processing. Most of what we know about functional interlaminar connectivity is from paired recordings in slice preparations which, due to cut axons, dramatically underestimate the rate of interlaminar connections. This leaves us with an incomplete understanding of how cortical circuits transform sensory information across layers. In this study, we use an optogenetic approach to bypass the issue of cut axons and comprehensively characterize functional excitatory interlaminar connections originating from cortical layers 2/3, 4, 5, and 6 onto both excitatory neurons and 3 inhibitory cell classes in layers 2/3, 4, 5, and 6. We are using transgenic mouse lines and conditional AAV vectors to restrict Channelrhodopsin-2 expression to excitatory cell populations within a single layer and to selectively label inhibitory cells of a particular class in mouse primary visual cortex. We then pair optogenetic stimulation of axon terminals with *ex vivo* intracellular recordings in excitatory neurons and somatostatin-, parvalbumin-, or vasoactive intestinal polypeptide-expressing inhibitory cells across cortical layers and measure the monosynaptic input strength from the stimulated cell population to the recorded cell. This enables us to compare postsynaptic responses between excitatory and inhibitory subtypes and cells recorded in different layers. We can thereby quantify the proportion of excitatory input from each cortical layer to each neuron subpopulation within and between layers. L4 provides most of its input to L2/3, and L2/3 provides strong input both within L2/3 and to L5. While extratelencephalic (ET) neurons in L5 primarily provide input to other cells in L5, L5 intratelencephalic neurons provide the majority of their input to L2/3. Inputs from L6 ET neurons are evenly distributed between L4, L5, and L6. Preliminary suggest input strength from a given layer to a particular inhibitory cell type depends strongly on both the input layer, the laminar location and the inhibitory subtype of the recipient cell. We also expect functional input strength to differ between excitatory cells and inhibitory cells and are further examining how each layer-specific population provides input to excitatory versus inhibitory cells across layers. Together, our results provide a more complete picture of the

relative distribution of excitatory synaptic input from each layer to distinct cell subpopulations across layers within a single cortical region.

Disclosures: M. Rossa: None. **E.M. Callaway:** None.

Poster

P474. Visual Cortex: Functional Architecture and Circuits

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Program #/Poster #: P474.08

Topic: D.07. Vision

Support: R25GM056929-22/23

Title: Correlation of brain-state related acetylcholine fluctuations and dendritic activity in mouse cortex.

Authors: *M. A. GALDAMEZ, E. NEYHART, C. L. SMITH, J. REIMER;
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Abstract: The integration of sensory feedforward and contextual feedback information is long thought to be key to adaptive cortical processing in varying environmental contexts. Within Layer 1 of the cortex, recipient apical dendrites of pyramidal cells integrate information from feedback projections with somatic activity. In addition, Layer 1 apical dendrites receive projections from interneuron subclasses and from subcortical neuromodulatory nuclei, including cholinergic axons from the basal forebrain. Previous *in vitro* studies have shown that acetylcholine modulates the excitability of Layer 1 dendritic tufts, and recent *in vivo* studies have demonstrated strong correlations between acetylcholine and brain states. However, the relationship between natural fluctuations in acetylcholine levels and dendritic activity *in vivo* remains unknown. Here we employed two-photon random access mesoscope (2P-RAM) imaging to simultaneously record acetylcholine levels and Layer 1-5 cortical activity using the fluorescent acetylcholine sensor, GACh4.3, and a genetically encoded calcium indicator, GCaMP7f, in awake mice. Our initial results suggest that acetylcholine levels are more correlated with dendritic excitation than somatic activity. We will perform acetylcholine and calcium imaging in Layer 5 Cre-dependent mouse lines to further characterize the natural spatiotemporal dynamics between local acetylcholine levels and dendritic activity. Our preliminary results are consistent with the idea that acetylcholine fluctuations gate feedback integration with implications for cortical plasticity and processing.

Disclosures: M.A. Galdamez: None. **E. Neyhart:** None. **C.L. Smith:** None. **J. Reimer:** None.

Poster

P515. Cerebellum: Human Studies

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P515.01

Topic: E.02. Cerebellum

Title: A multiplexed gradient in human cerebellum of macromolecules, function, and development

Authors: *J. GOMEZ[1], X. LIU[2], F. D'OLEIRE UQUILLAS[1], Z. ZHEN[2];
[1]Princeton Neurosci. Inst., Princeton, NJ; [2]Beijing Normal Univ., Beijing, China

Abstract: Despite its role in mediating a wide range of cognitive functions, and its implication in disorders such as autism, how the basic tissue and functional properties of the cerebellum are organized across development is unknown. Combining multiple large datasets, we demonstrate in the human cerebellum multiple gradients across the cerebellum with coordinated structural, functional, and developmental properties. First, in a large group of adults from the Human Connectome Project (HCP, n=1026), we find a static U-shaped tissue gradient in adults whereby dorsal cerebellar lobules (the most distant from the brainstem) show the least myelination compared to ventral lobules. We hypothesized that this adulthood myelin gradient should predict a developmental gradient across childhood, under a model in which the cerebellum follows a similar rule to cortex whereby highly myelinated structures (e.g., sensory cortex) myelinate early in development. Employing the developmental HCP dataset, we validate this prediction in n=625 individuals between the ages of 8 and 20. We find that dorsal cerebellar lobules undergo the most dramatic tissue growth across childhood, resulting in a smooth U-shaped gradient of tissue growth rates around the cerebellum ($F(24,5449) = 7.21$, $p < 0.001$). To investigate if these structural changes have any bearing on function, we ask if potential myelin growth sculpts the intrinsic functional properties of the cerebellum, with the strong a priori hypothesis that functional development should also follow a similar U-shaped gradient. Using resting-state data in the same individuals, we find that those lobules that undergo the most tissue growth demonstrated the most dramatic decrease in low-frequency fluctuations from a frequency analysis of the resting-state time series ($F(24, 5367) = 6.36$, $p < 0.001$), suggesting the structural development may serve to dampen random fluctuations. Importantly, the rate of functional development in different lobules forms a U-shaped gradient across the cerebellum that mirrors the tissue development gradient. Applying quantitative MRI in the cerebellum for the first time, we show the observed tissue gradient may have a unique macromolecular source compared to neocortex. Overall, across multiple datasets, we provide evidence for the existence of a coordinated multilayer gradient in the human cerebellum. These findings significantly advance our understanding of how the human cerebellum develops both structurally and functionally from childhood to adulthood. The existence of this multiplexed gradient provides a useful biomarker for future research to classify atypical development of the cerebellum.

Disclosures: J. Gomez: None. **X. Liu:** None. **F. d'Oleire Uquillas:** None. **Z. Zhen:** None.

Poster

P515. Cerebellum: Human Studies

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P515.02

Topic: E.02. Cerebellum

Support: NS100543

Title: Myogenic evoked responses to cerebellar dentate nucleus deep brain stimulation in human subjects

Authors: *F. PUCCI[1], M. SCHROEDEL[1], S. SAHOO[2], A. G. MACHADO[3], K. B. BAKER[4]; [1]Cleveland Clin. Fndn., Cleveland, OH; [2]Cleveland Clin. Lerner Col. of Med., Cleveland, OH; [3]Ctr. Neurolog. Restoration, [4]Dept. of Neurosci., Cleveland Clin., Cleveland, OH

Abstract: We report here evidence for myogenic evoked potentials (EPs) in response to electrical stimulation of the cerebellar dentate nucleus (DN) in a limited cohort of human subjects. Deep brain stimulation (DBS) of the deep cerebellar nuclei is under active investigation as a potential therapy for stroke recovery, TBI, and psychiatric conditions. Patients with a history of cerebral stroke affecting upper extremity (UE) motor function were enrolled in the *Electrical stimulation of the dentate nucleus* (EDEN) trial and underwent surgical implantation of a DBS system in the DN ipsilateral to the affected extremity. Data from two adult human subjects are reported here. The dentate nucleus was stimulated at a low frequency (3-6 Hz) with either single pulses or a train of three (pulse width 90 μs, amplitude 2-6 mA) in an EP paradigm. Electromyography (EMG) was recorded from muscle groups in the UE. The EMG signal was filtered from 10-1000 Hz, rectified, and a moving root mean square was taken. Segments of data time-locked to DN stimulation were excerpted and averaged to generate an EP. The experiment was performed with the affected UE both at rest and during a motor block-tracking task. Robust EPs could be elicited by DN stimulation in UE muscles ipsilateral to DN stimulation during the motor task. In a smaller subset of trials, evoked potentials could be elicited at rest. The waveform was composed of multiple peaks: a short latency (mean 32.5 ms) increase in EMG power indicating muscle activation, a subsequent inhibition of EMG activity below baseline (58.2 ms), and a second increase in power (106.4 ms). DN stimulation therefore can produce bidirectional modulation of muscle activity. EPs elicited during the motor task were then re-generated by subsampling the data and sorting it by baseline EMG power. In this way, one can compare the EP waveform stratified during active contraction and relaxation of the muscle belly according to EMG. The normalized positive going peaks were 2.8 and 1.3 times larger, respectively, during periods of relatively greater electrophysiological muscle activity. The short latency of these peaks in EMG power suggest an alternative path to the dentatothalamocortical tracts. One

hypothesis may be DN output to the reticulospinal pathway seen in animal models. Our data furthermore show that motor output from the DN to the UE muscle is somehow gated by the baseline motor state of the muscle, with evoked potentials being greatest during putative muscle contraction. This is an early demonstration of DN stimulation evoked myogenic potentials in humans, and further experiments are needed to determine the mechanism and physiological or clinical relevance of this EP paradigm.

Disclosures: F. Pucci: None. **M. Schroedel:** None. **S. Sahoo:** None. **A.G. Machado:** None. **K.B. Baker:** None.

Poster

P515. Cerebellum: Human Studies

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Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P515.03

Topic: E.02. Cerebellum

Support: NINDS UH3-NS100543

Title: Movement-related Cortico-cerebellar Interactions In Chronic Post-stroke Patients

Authors: *R. GOPALAKRISHNAN[1], D. A. CUNNINGHAM[5], M. SCHROEDEL[2], B. CAMPBELL[2], E. B. PLOW[3], K. B. BAKER[4], A. G. MACHADO[1];
[1]Ctr. for Neurolog. Restoration, [3]Biomed. Engin., [4]Dept. of Neurosci., [2]Cleveland Clin., Cleveland, OH; [5]Physical Med. and Rehabil., Case Western Reserve Univ., Cleveland, OH

Abstract: Novel therapies aimed at promoting post-stroke rehabilitation are in great need. Our group is investigating deep brain stimulation (DBS) of the dentatothalamocortical pathway as a method to modulate cerebral cortical activity and promote long-term motor recovery. Given the anticipated lack of acute behavioral effect(s) associated with the intervention, a key component of our strategy is to understand the connectivity dynamics between the surgically-targeted cerebellar dentate nucleus (DN) and the contralateral perilesional cerebral cortex. This is an essential first step to develop biomarkers that could facilitate therapeutic programming. Here, we present electrophysiological data collected during a motor control task. Ten patients undergoing DN DBS for post-stroke rehabilitation underwent scalp electroencephalography (EEG), local field potentials (LFP) from the externalized, 8-contact DN DBS lead, and surface electromyography (EMG) during a visuo-motor grip block tracking task. An average of 76 block tracking trials were collected with effort set to 20% maximal voluntary contraction. Each trial had a movement onset (grip squeeze), isometric grip hold and movement offset (grip relax). Movement related oscillations in the cerebellar DN were highly correlated with perilesional cortex oscillations during movement onset especially in the low beta band, with classic Event-related Desynchronization and Synchronization (ERD/ERS) phenomena representing motor

facilitation and inhibition, respectively. Less impaired subjects show greater ERD in both DN and perilesional cortex (low beta band), compared to high impaired subjects. During the isometric hold period of the tracking task, we observed significant coherence in the low beta band between the DN and the perilesional cortex. This cortico-cerebellar coherence was significantly positively correlated with task accuracy. The results show first ever in-human electrophysiological evidence for communication between the cerebellar DN and perilesional cortex in the low beta band during motor control. Future work will focus on how these metrics could facilitate therapeutic programming and evaluate DN DBS therapy.

Disclosures: R. Gopalakrishnan: None. **D.A. Cunningham:** None. **M. Schroedel:** None. **B. Campbell:** None. **E.B. Plow:** None. **K.B. Baker:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intellectual property and distribution rights in Enspire DBS Therapy Inc. F. Consulting Fees (e.g., advisory boards); Scientific advisory board of Enspire DBS Therapy. **A.G. Machado:** A. Employment/Salary (full or part-time):; Chairman, Neurological Institute Professor, Department of Neurosurgery Cleveland Clinic. 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.; Boston Scientic Intrepid DBS trial. Funds to the institution Enspire DBS. Funds to the Institution. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); none. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); none. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Enspire DBS - distribution rights of Intellectual Property Cardionomics - distribution rights of Intellectual Property. F. Consulting Fees (e.g., advisory boards); Boston Scientific Abbott Enspire DBS.

Poster

P515. Cerebellum: Human Studies

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P515.04

Topic: E.02. Cerebellum

Support: NIH Grant R01 HD040289

Title: Leveraging temporally-specific reinforcement to train a sequential movement in cerebellar ataxia

Authors: *R. A. MOONEY[1], D. CAO[1], P. A. CELNIK[1], A. J. BASTIAN[2,1];
[1]Johns Hopkins Univ., Baltimore, MD; [2]Kennedy Krieger Inst., Baltimore, MD

Abstract: Damage to the cerebellum causes ataxia, a movement disorder characterized by impaired motor coordination. People living with ataxia exhibit dysmetria and prolonged trajectory paths when making upper limb movements, which can lead to difficulty in performing activities of daily living. Previous studies have shown that reinforcement mechanisms, driven by simple binary signals (e.g., success or failure), can be leveraged to reduce elements of ataxia during discrete point-to-point reaching. Yet, many activities of daily living require sequential movements, such as reaching for, drinking from and then replacing a glass of water. Binary feedback after a sequential movement can be difficult to interpret - it does not tell the individual which segments of the sequence are faulty. Here we examined whether people with ataxia could use segment-specific binary feedback to learn a sequential movement. Participants learned a 3-dimensional sequential arm movement in virtual reality over four consecutive days. They were asked to make a continuous arm movement that traversed four invisible segments marked by a starting point, three via points and an end point. Their hand position was represented by a virtual ball. For each trial, we calculated the average distance error between their hand position and the invisible segments. Reinforcement was given via binary feedback based on whether each segment's error was above or below the average error of the previous five trials. On each day, five trials with no binary feedback were performed before and after 60 trials of reinforcement training. In a first experiment, we compared learning with binary feedback delivered immediately after each segment (temporally-specific) or after the entire sequence was complete (terminal) in healthy young subjects ($n = 12$ per group). Both groups improved performance on the whole movement by the end of day four (both $p < 0.001$). However, the temporally-specific group learned significantly more than the terminal group, reducing error by ~75% versus ~50%, respectively ($p < 0.001$). Next, we determined whether temporally-specific reinforcement could be used to train the same sequential movement in a group ($n = 10$) of people with ataxia. We found that the ataxia group was able to reduce error by ~50% by the end of day four ($p = 0.001$). These findings indicate that segment-specific reinforcement, provided in close temporal proximity to execution, can be leveraged to train a sequential upper limb movement in people with ataxia. Our results have important implications for designing effective reinforcement paradigms to optimize motor learning in the context of clinical rehabilitation.

Disclosures: R.A. Mooney: None. **D. Cao:** None. **P.A. Celnik:** None. **A.J. Bastian:** None.

Poster

P515. Cerebellum: Human Studies

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P515.05

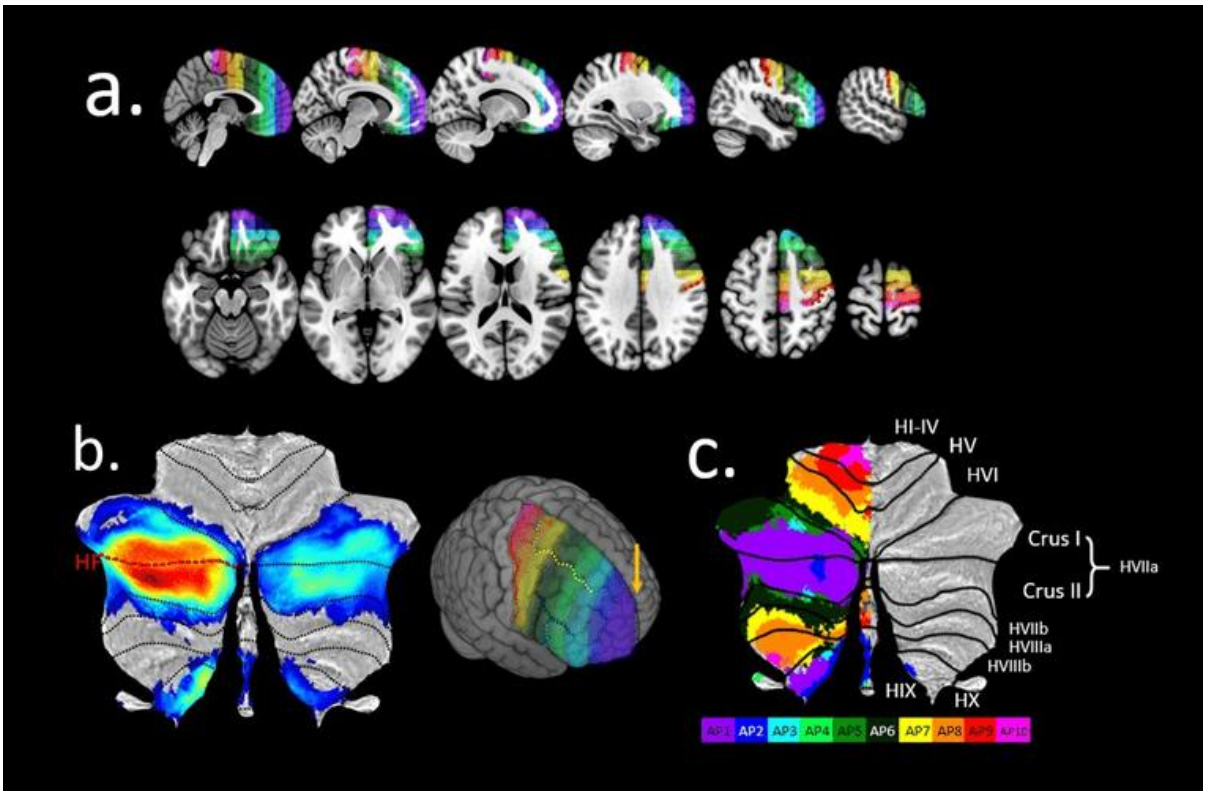
Topic: E.02. Cerebellum

Support: BBSRC Grant BB/S009477/1

Title: Topographically organised frontal cortex maps in the human cerebellar cortex.

Authors: *M. LONGLEY, M. DANVERS, N. RAMNANI;
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Abstract: The cortico-cerebellar system is one of the largest in the primate brain and connectivity with the frontal cortex is particularly well-understood. Prefrontal area 46 in anterior parts of the frontal lobe is connected with cerebellar cortical lobule HVIIA. However, some cerebral cortical areas have discontinuous representations in the cerebellar cortex. Primary motor cortex (area 4) is interconnected with lobules HIV-HVI and lobules HVIIB-HVIII, either side of lobule HVIIA (Kelly and Strick, 2003). Previous human resting state fMRI (rsfMRI) studies also show that spatially distinct cerebellar cortical areas form networks with the same areas in the cerebral cortex (Buckner et al, 2011; Guell et al., 2018; Xue et al. 2021).
The frontal lobe is functionally organised along an anterior-to-posterior axis (Ramnani and Owen, 2007; Badre and D'Esposito, 2009), and projections from the frontal lobe to subcortical structures are topographically ordered (Ramnani et al., 2006; Marquand et al., 2017). We tested the hypothesis that similarly, adjacent anterior-to-posterior segments of the frontal cortex are represented in adjacent areas of the cerebellar cortex.
We performed seed-based resting-state analysis (CamCAN dataset; N=80, 43 female; CONN Toolbox / SPM12 / Matlab) using a frontal cortex mask divided into 10 contiguous anterior-to-posterior sections (fig. 1a, AP1-AP10).
Consistent with previous work, we report evidence for three maps in the cerebellar cortex (fig 1b, c; cerebellar cortical flatmaps; SUIT). First, an area between lobule HIII and Crus I HVIIA (fig 1c). Second, an area from Crus II HVIIA to lobule HVIIIB. Third, within lobule HIX. We also report these to be topographically organised (fig 1b): anterior parts of the frontal cortex are represented immediately adjacent to and either side of HF, and within lobule HIX. More posterior segments of the frontal cortex are represented at increasingly distant areas from the HF in the first two maps. Our future work will investigate the ways in which each map interacts with the frontal cortex to support movement and cognitive control.



Disclosures: M. Longley: None. **M. Danvers:** None. **N. Ramnani:** None.

Poster

P515. Cerebellum: Human Studies

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P515.06

Topic: E.02. Cerebellum

Title: Fatigue perception: The role of the cerebellum

Authors: *A. CASAMENTO MORAN[1], R. A. MOONEY[1], V. S. CHIB[3], A. J. BASTIAN[4], P. A. CELNIK[2];
[1]Physical Med. and Rehabil., [2]Physical Med. & Rehab, Neurol, Johns Hopkins Univ., Baltimore, MD; [3]Biomed. Engin., Johns Hopkins Sch. of Med., Baltimore, MD; [4]KKI & Johns Hopkins, Baltimore, MD

Abstract: Fatigue is the subjective sensation of weariness, increased sense of effort, or exhaustion and is common in neurological illness. Despite its prevalence, we have a limited understanding of the neurophysiological mechanisms underlying fatigue because previous studies have investigated factors that impair motor function (e.g., reduced force capacity, also

known as fatigability) without addressing fatigue itself (i.e., the percept). Growing evidence suggests that the cerebellum, known for its role in motor control and learning, is a key structure involved in perceptual processing. However, the relationship between cerebellar activity and the perception of fatigue remains largely unexplored. Here, we perform two experiments to examine how cerebellar excitability is modulated after a fatiguing task and its association with both fatigue and fatigability. In 21 participants, we used a cross-over design to assess corticomotor excitability, cerebellar inhibition (CBI), and the participant's perception of fatigue before and after a fatiguing or a control protocol. Participants performed five trials of an isometric pinch between their thumb and index finger at 80% maximum voluntary capacity (MVC) until failure (force lower than 40% MVC; fatigue) or at 5% of maximum capacity for 30 s (control). We found reduced CBI after the fatigue protocol, indicating a reduction in cerebellar excitability. Interestingly, lower CBI correlated with a milder perception of fatigue but did not correlate with the time to task failure (i.e., fatigability). Furthermore, we found reduced M1 excitability after both protocols, with a greater reduction in the fatigue condition, a finding consistent with prior reports. These results suggest that cerebellar excitability after a fatiguing task may reflect a compensatory mechanism to minimize the perception of fatigue. To investigate the behavioral consequences of reduced cerebellar excitability after fatigue, we performed a follow-up experiment to examine the interaction between fatigue, CBI changes, and motor control. We recruited another 20 participants and measured CBI, perception of fatigue, and performance during a ballistic goal-directed task before and after the same fatigue and control protocols. Again, we found reduced CBI after fatigue and replicated our observation that lower CBI correlated with a milder perception of fatigue. In terms of performance, we found greater endpoint error and variability which correlated with reduced CBI after fatigue. Together, these results suggest that changes in cerebellar excitability after fatigue may modulate the perception of fatigue at the expense of motor control.

Disclosures: A. Casamento Moran: None. **R.A. Mooney:** None. **V.S. Chib:** None. **A.J. Bastian:** None. **P.A. Celnik:** None.

Poster

P616. Regulating Systems I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P616.01

Topic: F.05. Neuroimmunology

Support: NIA Grant R15AG052935

Title: Exercise duration differentially effects age-related neuroinflammation and hippocampal neurogenesis

Authors: M. G. CONNOLLY[1], S. R. BRUCE[2], *R. A. KOHMAN[2];
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Abstract: Regular exercise has many health benefits and may be particularly important with aging as exercise attenuates cognitive decline, neuroplasticity deficits, and immune dysregulation. The response to exercise varies as a function of frequency and length. However, research on the duration-dependent effects of exercise has focused primarily on young adults and less is known about the influence of exercise duration in the aged. The current study compared the effects of short-term and long-term running wheel access on hippocampal neurogenesis and neuroimmune markers in aged (19-23 months) male C57BL/6J mice. Aged mice were given 24-hour access to a running wheel for 14 days (short-term) or 51 days (long-term). Groups of non-running aged and young (5 months) mice served as comparison groups to detect age-related differences and effects of exercise. Long-term, but not short-term, exercise increased hippocampal neurogenesis as assessed by number of doublecortin-positive cells in the granular cell layer. Assessment of cytokines, receptors, and glial-activation markers showed the expected age-related increase compared to young controls. In the aged, exercise as a function of duration regulated select aspects of the neuroimmune profile. For instance, hippocampal expression of interleukin (IL)-33 was reduced and IL-10 increased only following long-term exercise. Whereas brain levels of IL-6 were reduced by both short- and long-term exercise. Additional findings showed that exercise does not modulate all aspects of age-related neuroinflammation and/or may have differential effects in hippocampal compared to brain samples. Overall, the data indicates that increasing exercise duration produces more robust effects on immune modulation and hippocampal neurogenesis.

Disclosures: M.G. Connolly: None. **S.R. Bruce:** None. **R.A. Kohman:** None.

Poster

P616. Regulating Systems I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P616.02

Topic: F.05. Neuroimmunology

Support: F32 HD097816-03
R01 MH52716-21

Title: The immune cell profile of the developing rat brain

Authors: *E. L. REINL, A. C. BLANCHARD, E. L. GRAHAM, M. M. MCCARTHY;
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Abstract: Peripheral immune cells (PICs) populate the brain and its surrounding meninges, contributing to the function and development of the CNS. In recent years, flow cytometric and single cell sequencing studies have described the immune cell composition of the adult rodent brain in healthy, diseased, and injured states, whereas studies of the developing brain have been vastly limited. Knowledge of the immune system within the developing brain is essential for understanding how these cells may contribute to normal developmental processes, and the induction of developmental disorders rooted in immune disruption. Our objective was to delineate the immune cell repertoire of specific brain regions including the hippocampus, hypothalamus, and cerebellum from developing rats of both sexes starting from embryonic day 20 (E20) to postnatal day 0 (PN0), PN7, and PN16. We used 11-color flow cytometric analysis to identify 10 unique cell types including microglia, T cells, B cells, NK cells, mast cells, neutrophils, and monocytes. We report four main findings. FIRST: immune cell counts within brain regions increased with age, this increase was primarily driven by microglia, and the percent of total immune cells that are peripherally derived decreased with age. At their peak at E20, PICs were most abundant in the cerebellum (40%), followed by hippocampus (20%) and hypothalamus (10%). SECOND: within the PIC compartment, monocytes dominated in all brain regions and at all ages, composing ~60% of PICs at E20 and decreasing to ~40% by PN7. Their subtype makeup changed with age. CD43hi (non-classical) monocytes made up an increasing proportion of the monocyte pool as animals developed, and HIS48hi (classical) monocytes decreased over the same period. Additionally, a novel monocytic cell type was discovered that decreased with age in all brain regions. These cells were negative for CD43 and HIS48, but like other myeloid cells, were CD45hi, CD11b+, CD172+, and, surprisingly, B220+. These cells may represent monocyte progenitors infiltrating from the bone marrow niches as was recently described in PMID 34083447. THIRD: T cell populations increased with age in all brain regions. Both CD4+ (helper) and CD8+ (killer) populations increased, but helper T cells increased at a greater rate, such that the CD4/CD8 ratio flipped by PN7. FOURTH: mast cells were more populous early in development, with numbers peaking at PN7 and dropping to near zero by PN16, and they were much more numerous in the hippocampus, followed by the hypothalamus, and were sparse in the cerebellum. Our findings implore investigators to look beyond microglia and toward PICs as a source of immunomodulation in the developing brain.

Disclosures: E.L. Reinl: None. **A.C. Blanchard:** None. **E.L. Graham:** None. **M.M. McCarthy:** None.

Poster

P616. Regulating Systems I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P616.03

Topic: F.05. Neuroimmunology

Support: NIH/NCATS Grant UL1TR001073
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NIH/NIMH Grant R21MH126501
NIH/NIMH Grant R01MH101479
NIH/NIMH Grant R01MH095807
NIH/NIMH Grant R01MH120601

Title: Adrenergic Attenuation of Immune Activity is Associated with Anhedonia in Adolescents

Authors: *T. N. B. NGUYEN[1], B. A. ELY[1], D. PICK[1], S. KIM-SCHULZE[2], C. ALONSO[1], V. GABBAY[1,3];
[1]Psychiatry and Behavioral Sci., Albert Einstein Col. of Med., Bronx, NY; [2]Human Immune Monitoring Ctr., Icahn Sch. of Med. at Mount Sinai, New York, NY; [3]Nathan S. Kline Inst. for Psychiatric Res., Orangeburg, NY

Abstract: ***Background***: While the link between peripheral inflammation and psychiatric symptoms has been supported, the role of immune-modulating processes by the adrenergic nervous system in psychopathology remains unclear. Here, we sought to investigate the β_2-agonist clenbuterol (CBL) as a promising agent to model adrenergic-driven immune attenuation following *ex vivo* immune activation with lipopolysaccharide (LPS). Further, we explored the relationships between CBL-modulated cytokine levels and psychiatric measures in our adolescent participants. ***Methods***: We conducted semi-structured interviews to evaluate 130 participants. Depression, anxiety, and anhedonia levels were measured with the BDI, MASC, and TEPS questionnaires. Fasting whole-blood samples were acquired and stimulated with LPS (0.1 μg/mL) in the presence and absence of CBL (10^{-6} M) for 6 hours. Supernatants were collected and subjected to Luminex multiplex assay for 41 cytokines. Cytokine levels were compared between 3 conditions: Control, LPS, and LPS+CBL. Then, we utilized an exploratory factor analysis (EFA) to dimensionally reduce cytokine data from the 3 experimental conditions. Spearman correlations between each immune factor and psychiatric symptoms were examined, controlling for age, sex, and BMI. The statistical significance of all associations was assessed after Bonferroni correction for multiple comparisons. ***Results***: Levels of 9 cytokines were significantly attenuated by CBL treatment compared to LPS alone. Across 3 conditions, EFA reduced 41 cytokines into 5 orthogonal factors, and a similar factor was identified to be significantly correlated with anticipatory anhedonia after Bonferroni correction. Specifically, correlations were found between anticipatory anhedonia and a) Control Factor (F) 4 (rho = -0.35, $p = 3.6 \times 10^{-4}$), b) LPS F3 (rho = -0.36, $p = 2.6 \times 10^{-4}$), and c) LPS+CBL F3 (rho = -0.39, $p = 7.4 \times 10^{-5}$). In addition, LPS+CBL F3 significantly correlated with consummatory anhedonia (rho = -0.36, $p = 3.3 \times 10^{-4}$). No significant correlation was found between any immune factor and depression or anxiety. Post-hoc analysis contrasting cytokine relationships between LPS+CBL and LPS conditions revealed differences in inter-cytokine correlation structures, possibly explaining CBL's effects on the relationships between peripheral immune response and anhedonia sub-components. ***Conclusions***: Findings support our hypothesis that CBL attenuates inflammatory effects thought to underlie psychiatric conditions in youth. Following data-driven analyses, distinctive associations between adrenergic-modulated immune activity and dimensional anhedonia were identified.

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Poster

P616. Regulating Systems I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P616.04

Topic: F.05. Neuroimmunology

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GX19-28347X
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ISF 1709/19

Title: A cell atlas and live imaging tools to study the choroid plexus blood-CSF barrier

Authors: *N. DANI[1], R. H. HERBST[3], F. B. SHIPLEY[2], C. MCCABE[3], H. XU[2], G. S. GREEN[4], K. KAISER[5], J. P. HEAD[2], D. DIONNE[3], V. BRYJA[5], O. ROZENBLATT-ROSEN[3], C. I. MOORE[6], N. HABIB[4], M. L. ANDERMANN[7], A. REGEV[3], M. K. LEHTINEN[2]; [1]Boston Children's Hosp., Quincy, MA; [2]Boston Children's Hosp., Boston, MA; [3]Broad Inst., Cambridge, MA; [4]Hebrew Univ. of Jerusalem, Jerusalem, Israel; [5]Masaryk Univ., Brno, Czech Republic; [6]Brown Univ., Providence, RI; [7]Beth Israel Deaconess Med. Ctr., Boston, MA

Abstract: The choroid plexus (ChP) is found in each of the four brain ventricles and functions as a distributed blood-CSF barrier system. Cells of the choroid plexus produce CSF and secrete age-specific factors that support brain development and health across lifespan. The ChP also functions in host-defense as a neuroimmune barrier that gates the entry of immune cells into the brain. More recently, the SARS-CoV-2 virus is reported to show neurotropism for the ChP, which potentially contributes to neurological symptoms associated with the disease. Now more than ever, there is a pressing need to understand this vital brain barrier. However, there have been historical setbacks presented by an incomplete characterization of the ChP and the lack of tools to study the system *in vivo* using genetically tractable models. Recently, we applied single-cell and single-nucleus transcriptomics to generate a cell atlas of the choroid plexus in the developing (embryonic day (E)16.5), adult (4 months) and aging (20 months) mouse brain. We find stable, ventricle- and age-specific gene expression patterns in ChP cells including epithelial, endothelial, mesenchymal, neuronal, glial and immune cell types. Using trajectory analysis and lineage tracing approaches, we identify and map the location of a common progenitor pool that gives rise to choroid plexus epithelial cells and neurons in the developing mouse brain. Further,

mapping ligand and receptor gene expression across brain ventricles and ages, we identify extensive cellular crosstalk within the ChP, with mesenchymal cells functioning as a signaling hub that communicates with all cell types. Guided by transcriptional insights from the cell atlas, we can now genetically target ChP cells (CRISPR) and use recently innovated live imaging strategies to visualize their activity in explants, *ex vivo* and *in vivo*. For example, by two-photon calcium imaging (GCaMP6f) in awake behaving adult mice, we visualize apocrine secretion into the CSF evoked by 5-HT2C serotonin receptor agonists. Similarly, by tracking *Cx3cr1-GFP* expression in choroid plexus macrophages, we identify homeostatic, inflammation and tissue-damage evoked responses *in vivo*. Overall, by combining transcriptional insights from the cell atlas and the live imaging technologies, we can investigate cellular and molecular mechanisms of ChP cell types during brain development, host-defense, aging and in neurologic disease models.

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Poster

P616. Regulating Systems I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P616.05

Topic: F.05. Neuroimmunology

Title: Sex differences in stroke dysbiosis in a rodent model of focal cortical ischemia

Authors: *E. DANIELE[1], N. SACHEWSKY[1], J. COPELAND[3], E. Y. SCOTT[2], L. UMBRIO[1], M. FAIZ[1];
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Abstract: Stroke is a devastating neurological disease which effects males and females differently across the lifespan (Haast *et al.,* 2012). Stroke related changes in gut microbiota, stroke dysbiosis, have been shown to play important role in stroke outcomes (Singh *et al.,* 2016). Given that there are sex differences in gut microbial composition throughout the lifespan (Kim *et al.,* 2020), there has been little study of stroke dysbiosis in males and females in rodent models. The aim of this study was to characterize whether there are sex differences in the trajectory of stroke dysbiosis in the acute and chronic phase of stroke injury using a rodent model of focal cortical ischemia. We collected feces from 8-week-old male and female C56 BL6/J mice prior to (D0) and at acute (D4) and chronic timepoints (D10, D20, D60) following ET-1 stroke injury. Fecal gDNA was extracted and subjected to 16s V4 hypervariable region sequencing. We

observed that females exhibit a significant increase in alpha species diversity (Shannon Entropy) at post-stroke D60 compared to D4 (Kruskal Wallis, $p<0.05$), whereas males did not exhibit such change (Kruskal Wallis, > 0.05). When comparing beta species diversity (Bray Curtis Dissimilarity), we observed that both males and females exhibit stroke dysbiosis at D4 (PERMANOVA, $p < 0.05$), however males and females differed in stroke dysbiosis in the chronic phase of injury. Strikingly, females exhibited a return to D0 composition at D20 (PERMANOVA, $P > 0.05$), whereas males remained significantly different from their D0 composition at D20 and D60 (PERMANOVA $p < 0.05$). Next, we studied differentially abundant bacteria in the gut microbiota in males and females following ischemic stroke injury using a linear discriminant effect size (LEFSE) analysis on the relative abundance of operational taxonomic units (OTUs). Interestingly, we did not observe LEFSE hits when examining the relative abundance of OTUs in female animals, however we observed that males exhibited an increased abundance of *Lactobacillaceae* OTUs at D0, *Verrucomicrobiaceae* OTUs at D4 and *Dehalobacteriaceae* OTUs at D10 (Kruskal Wallis, $p < 0.05$). To our knowledge, this study is the first to characterize stroke dysbiosis in the acute and chronic phase of injury in both males and females. These findings will support future studies of how sex differences in the gut-microbiota-brain-axis contribute to stroke outcomes.

Disclosures: E. Daniele: None. **N. Sachewsky:** None. **J. Copeland:** None. **E.Y. Scott:** None. **L. Umbrio:** None. **M. Faiz:** None.

Poster

P616. Regulating Systems I

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Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P616.06

Topic: F.05. Neuroimmunology

Title: Quantification of unmyelinated nerve fibers in electron microscopy images using deep learning

Authors: V. MANNAM[1], V. TOTH[2], *E. H. CHANG[2,3], T. ZANOS[2,3];
[1]Dept. of Electrical Engin., Univ. of Notre Dame, Notre Dame, IN; [2]Inst. for Bioelectronic Med., Feinstein Inst. For Med. Res., Manhasset, NY; [3]Zucker Sch. of Med. at Hofstra/Northwell, Hempstead, NY

Abstract: The vagus nerve is a mixed nerve containing sensory and motor fibers that carry signals between the brain and body. Selective targeting of different fiber subsets is a major goal of neuromodulation strategies, including vagus nerve stimulation (VNS). Improved methods for identifying and quantifying specific fiber types within the vagus nerve (such as A, B, and C-fibers) would provide important insights for selective neuromodulation at the ultrastructural level and enable more realistic in-silico testing of different VNS parameters. While larger myelinated

A- and B-fibers are straightforward to identify, most of the fibers in the vagus nerve are unmyelinated sensory C-fibers that are difficult to identify due to their small size and poor contrast in most imaging modalities. Traditional methods have failed to provide quantitative information about unmyelinated fibers, which number in the thousands, to tens of thousands, depending on the species studied. In this study, we trained a deep learning (DL) based convolutional neural network (CNN) to identify and quantify unmyelinated C-fibers in the mouse cervical vagus nerve. Cross-sections from wildtype C57BL6 mice were processed with glutaraldehyde-paraformaldehyde fixation, followed by 1% osmium tetroxide, then stained in uranyl acetate. Ultra-thin sections were imaged with a transmission electron microscope (FEI) and UltraScan 4000 imaging system (GATAN). These TEM images were used as input images for training the model. Our trained DL model has instance segmentation architecture that can provide the spatial distribution (mask) and count of type-C unmyelinated fibers simultaneously for a given test image. We validated the fine-tuning of the DL model with various hyper-parameters (learning rate, batch size, number of iterations) for accurate type-C fiber detection. Generation of the target images requires intensive manual annotations; therefore, we limited our training dataset to a subset of our dataset. We incorporated a rapid inference-based image denoising network on the input images that enhanced the contrast information during the pre-processing step. Our trained model detects more than 50% type-C fibers for a given test image. By adding additional target images, we expect our model will identify more than 75% type-C fibers, in addition to providing quantitative measurements such as average area, the radius of fibers, and their spatial distribution within the nerve based on segmentation results. The demonstrated novel method implements an automated pipeline to quantify unmyelinated fibers from mouse vagus nerve TEM images, but it may also be applied to other nerves and species of interest.

Disclosures: V. Mannam: None. **V. Toth:** None. **E.H. Chang:** None. **T. Zanos:** None.

Poster

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Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P616.07

Topic: F.05. Neuroimmunology

Support: UTMB Institute for Human Infections and Immunity (FL, RS)
NIH R01MH124351 (FL)
NIH R01NS106597 (RS)
NIH T32GM008280 (NMD)

Title: Cerebral Malaria Induced Neuronal Hyper-Excitability Rescued via TNF signalling modulation:Potential for New Therapeutics

Authors: N. M. DVORAK[1], N. D. DOMINGO[2], C. M. TAPIA[1], ***R. STEPHENS**[3], F. LAEZZA[1];
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Abstract: Cerebral malaria (CM), caused by infection *with Plasmodium spp.* parasites, kills an estimated 425,000 people per year. While seizures increase the likelihood of death from CM, mechanisms driving seizures and the neuroinflammation seen in malaria are poorly understood. In this hyperinflammation driven model of CM, systemic erythrocyte-tropic *P. chabaudi* infection in IL-10 deficient animals, an excess of tumor necrosis factor (TNF) driven by absence of regulatory cytokine, IL-10, reproduces many symptoms of CM. In CM rodent models, TNF drives excess coagulation, blood brain barrier disruption, gliosis and epileptiform-like activity in the hippocampal CA1 region. As the voltage-gated Na+ (Nav) channel Nav1.6 is the primary molecular determinant of CA1 pyramidal neuron excitability, we postulated that molecular mechanisms underlying TNF-induced neuroinflammation in CM could be mediated via signaling pathways targeting the Nav1.6 channel. Using a high-throughput screening of kinase inhibitors, we previously showed that Janus kinase-2 (JAK2) signaling downstream of TNF leads to phosphorylation of fibroblast growth factor 14 (FGF14), a regulatory accessory protein of Nav1.6. Building on these results, we sought to determine the contribution of the JAK2 pathway to systemic infection-driven hippocampal alterations in CM. Using whole-cell patch-clamp electrophysiology, we now show that TNF, induced during CM, leads to an increase in intrinsic firing of CA1 pyramidal neurons, which is in line with previously reported epileptiform-like activity in CM. We also show that this increase in basal firing of CA1 hippocampal neurons can be prevented via *in vivo* genetic silencing of FGF14. Furthermore, small molecules targeting either JAK2 or the downstream FGF14:Nav1.6 channel complex can reverse the increased excitability of CA1 hippocampal neurons induced by TNF. Thus, pharmacologically targeting the TNF/JAK2 downstream FGF14:Nav1.6 channel complex may prove a viable therapeutic strategy to reduce epileptic seizures and decrease risk of death from hyperinflammatory CM.

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Poster

P616. Regulating Systems I

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National Institute for Health Research (NIHR)
Imperial Biomedical Research Centre (BRC)
MRC (grant number MR/N014103/1)

Title: Microbe-immune crosstalk: evidence that T cells influence the brain metabolome

Authors: *M. GREEN[1], G. CASPANI[3], J. SWANN[3], J. A. FOSTER[2];
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Abstract: The gut-brain axis describes a unique paradigm in which the intestinal microbiome acts as an important determinant of brain development, neurobiology, and behaviour. However, the mechanistic links connecting commensal microbes to neurological outcomes remain incompletely understood. T-lymphocytes, a key cellular component of the adaptive immune system, have recently emerged as an important mediator of this microbiota-brain communication, given their ability to interface with both the intestinal lumen and brain parenchyma at distinct lymphatic barriers. This study aimed to determine the impact of T cell deficiency on various “nodes” in the microbiota-brain axis, including compositional and functional microbiota characteristics as well as brain metabolites. We collected fecal and cecal samples from male and female T cell receptor (TCR) deficient (*TCRβ-/-δ-/-*) and C57Bl/6 wild type (WT) mice at postnatal day 17, 24, 28 and 84. Brain tissue samples including hippocampus, hypothalamus, and cortex were also collected. DNA extracts from feces and cecum were sequenced to obtain a microbiome profile based on 16S ribosomal rRNA. Additionally, we implemented 1H-NMR spectroscopy to extract a complete profile of metabolites within tissue, cecal and fecal samples. By examining both the microbiota (cecal and fecal) and the metabolome (cecal, fecal, gut and brain), we have identified key T-cell-related taxa and their metabolites that are integral to microbe-immune-brain signaling in the postnatal period. *TCRβ-/-δ-/-* mice display reduced microbial diversity, a unique microbiome composition and a distinct metabolic signature in both gut and brain compared to WT controls. Multiomic analysis also revealed that taxa of the family *Muribaculaceae* are strongly impacted by T cell deficiency and associated with specific metabolites, including the short-chain fatty acids butyrate and valerate in the gut. These were in turn associated with various neuroactive molecules, such as GABA and glycine, in the brain. Together, our findings demonstrate that T-cell related changes to the microbiome can directly affect levels of neuroactive metabolites in the CNS, with potential implications for host behaviour and neurodevelopment. Furthermore, these results align with previously observed behavioural deficits in *TCRβ-/-δ-/-* mice, suggesting a molecular basis for an altered neurodevelopmental trajectory in absence of adaptive immune signaling. These novel findings offer insight into the role of T cells in mediating microbe-brain crosstalk, in a dialogue that is essential for maturation of a normal brain and gut metabolome throughout postnatal life.

Disclosures: M. Green: None. **G. Caspani:** None. **J. Swann:** None. **J.A. Foster:** None.

Poster

P616. Regulating Systems I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P616.09

Topic: F.05. Neuroimmunology

Support: NIH Grant R01DA0462582

Title: The beneficial impact of IRF7 in human immunodeficiency virus-1 transgenic rats

Authors: *N. KOTA[1], M. VIGORITO[2], V. KRISHNAN[3], S. CHANG[3];
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Abstract: Interferon regulatory factors (IRFs) are a family of transcription factors that drive anti-viral responses. One member of this family, IRF7, is elevated in the brains of human immunodeficiency virus-1 transgenic (HIV-1Tg) rats. The purpose of this study is to investigate how IRF7 beneficial activity in response to HIV by comparing the differential expression of IRF7's associated molecules between HIV-1Tg and F344 (control) rats in the prefrontal cortex, striatum, and the hippocampus. This study utilized the CLC Genomic Workbench and the Ingenuity Pathway Analysis (IPA) from QIAGEN to analyze the differential expression between HIV-1Tg and F344 rats. The RNA sequencing data from GSE47474 was analyzed using CLC. Using the research findings from the QIAGEN Knowledge Base (QKB), 514 molecules associated with IRF7 were identified. Of this group, the molecules that overlapped with molecules from the differential expression dataset between HIV-1Tg and F344 rats ($p<0.05$) was identified, giving 29 molecules which include, but are not limited to, IFNG, MAP3K8, PARP14, and TRAF1. The Core Analysis of the molecules with differential expression between HIV1-Tg and F344 rats (with IRF7, $p<0.05$) generated 532 canonical pathways (with $p<0.05$). Of these pathways, 39 were found to with z score > +/- 2, with a z score greater than zero indicating a positive correlation and a z score less than zero indicating a negative correlation. A comparison between these significant pathways and their counterparts in the Core Analysis without IRF7 indicated that IRF7 is engaged in several key immune related pathways, such as the Th2 pathway, which is involved in the formation of the humoral cell response. In the presence of IRF7 and its associated molecules, the z score for the correlation between HIV and cardiac-related pathways significantly decreases, suggesting that IRF7 may play a beneficial role to prevent cardiovascular disease in HIV patients. Most noteworthy, IRF-7 plays its greatest role in epigenetics by modifying the expression of proteins that are necessary for chromatin modification and transcriptional activation. This antiviral epigenetic activity by IRF7 may have implications for decreasing HIV viral replication. Taken together, these findings reveal that IRF7 has a profound beneficial impact on the body's response to HIV by increasing the immune response, decreasing HIV's risk factors for cardiac-related diseases, and decreasing HIV's potential to replicate. Future studies can investigate if IRF7's beneficial activity may have

therapeutic value in developing treatment for HIV patients by investigating the relevant canonical pathways.

Disclosures: N. Kota: None. **M. Vigorito:** None. **V. Krishnan:** None. **S. Chang:** None.

Poster

P616. Regulating Systems I

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Program #/Poster #: P616.10

Topic: F.05. Neuroimmunology

Support: NIMH ZIA MH001090

Title: Ccr2 monocytes repair cerebrovascular damage caused by chronic social defeat stress

Authors: *M. LEHMANN, J. SAMUELS, S. KIGAR, C. POFFENBERGER, M. HERKENHAM;
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Abstract: Immune surveillance of the brain plays an important role in health and disease. Peripheral leukocytes patrol blood-brain barrier (BBB) interfaces and respond to signals to engage appropriate targets during infection and injury. Classical $CCR2^{+}$ monocytes guided by chemoattractant signals respond to these insults and enter target tissue where they follow a temporally scripted pattern of pro- and anti-inflammatory activity leading to repair and return to tissue homeostasis. We have shown that chronic social defeat (CSD) causes blood-brain barrier dysfunction in the form of scattered disruptions of the cerebrovasculature. Here, we tracked monocyte trafficking to the vascular injury sites in *CCR2*$^{wt/rfp}$ reporter mice both during the CSD and following CSD cessation. We found that CSD for 14 days induced behavioral deficits in sociability coupled with microhemorrhages where plasma fibrinogen leaked into perivascular spaces and parenchyma. CSD had no effect on either the anatomical distribution or density of CCR2+ monocytes in the brain. However, if mice were given seven days to recover from CSD stress in the homecage environment, a substantial and selective elevation in numbers of adhered vascular CCR2+ cells was detected. Endothelial cell expression of the CCR2 ligands CCL7 and CCL12, but not CCL2, was found in the CSD recovery group. Adhered CCR2+ cells were mostly the non-classical, anti-inflammatory $Ly6C^{lo}$ type, and they were shown by confocal microscopy to phagocytose fibrinogen in perivascular spaces. In CCR2-deleted *CCR2*$^{rfp/rfp}$ mice, fibrinogen levels remained elevated in recovery. Fibrinogen infused intracerebroventricularly induced CCR2+ cells to adhere to the vasculature in *CCR2*$^{wt/rfp}$ but not *CCR2*$^{rfp/rfp}$ mice. We hypothesize that peripheral CCR2+ monocytes play little role during chronic psychosocial stress but have restorative functions mediating vascular repair in stress recovery.

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Poster

P616. Regulating Systems I

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Program #/Poster #: P616.11

Topic: F.05. Neuroimmunology

Support: Conte - NIH # 5P50MH112491-05
Cure AD Understanding the consequences of non-coding AD risk alleles on microglia function

Title: Distinct stromal niches orchestrate neonatal lymphopoiesis at the borders of the brain

Authors: *A. J. WALKER[1,4,6], T. M. FISHER[2], H. J. BARR[2,5], S. E. MARSH[7,6], V. H. GAZESTANI[6], L. DISSING-OLESEN[1], T. R. HAMMOND[2,5,6], B. A. STEVENS[3,5,6,8]; [1]F.M. Kirby Neurobio. center, [3]Harvard Med. Sch. Neurobio., [2]Boston Children's Hosp., Boston, MA; [4]Immunol., [5]Harvard Med. Sch., Boston, MA; [6]Stanley Ctr. for Psychiatric Research, Broad Inst., Cambridge, MA; [7]Neurobio., Boston Children's Hospital/Harvard Med. Sch., Boston, MA; [8]Howard Hughes Med. Institute, Boston Children’s Hosp., Boston, MA

Abstract: Brain border interfaces including the meninges - and the immune populations that reside there - serve as key intermediaries in neuroimmune communication in the healthy adult and diseased central nervous system (CNS). However, much less is known about how meningeal immune cells develop, the stromal niche that shapes their function, and their crosstalk with the developing brain.We set out to map the development of the meningeal immune and stromal compartment in the postnatal mouse. Surprisingly, we uncovered a wave of B cells that colonize the dural meninges shortly after birth and largely disappear by adulthood. Single cell RNA sequencing (scRNA-seq) demonstrated that early life meningeal B cells recapitulate Pro, Pre, and immature B cell stages and are transcriptionally similar to B cells found in the overlying calvarium bone marrow. Given the intimate anatomical relationship between the calvarium bone marrow and the dura, we next aimed to define the stromal niches that orchestrate lymphopoiesis across this bone-meninges interface. Our scRNA-seq data revealed diverse populations of fibroblasts in the dura that produce canonical hematopoietic niche factors but are largely transcriptionally distinct from stromal populations that support hematopoiesis in the calvarium bone marrow. We found that large clusters of B cells associate with defined populations of fibroblast-like cells lining the dural meningeal sinuses, and that specific ablation of niche factors from meningeal fibroblasts disrupts B cell localization to this peri-sinus niche. Trans-calvarial two-photon imaging of neonatal mice revealed dynamic movement of B cells between the calvarium and the dura after disruption of niche factors, suggesting that these molecularly and

spatially defined niches may coordinately regulate lymphopoiesis in early life.This data provides critical insight into early life B cell development and underscores a general feature of the meninges as a functional B cell niche. Ongoing studies aim to explore the role of this fibroblast niche in B cell driven CNS diseases such as multiple sclerosis and CNS leukemia.

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Poster

P625. Respiratory Control II

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Program #/Poster #: P625.01

Topic: E.08. Respiratory Regulation

Support: Louis and Harold Price Foundation
H & H Evergreen Foundation
J. Yang Family Foundation

Title: Cervical spinal electrical epidural stimulation (EES) induced phase resetting of respiration

Authors: *Y. ZHOU[1], R. HUANG[2], J. WORRELL[2], E. GARNER[2], S. WANG[2], T. TOMSEY[3], B. XU[2], E. L. GALER[2], S. TAVAKOL[3], M. DANESHVAR[4], T. LE[2], H. V. VINTERS[2], N. SALAMON[1], D. L. MCARTHUR[1], M. R. NUWER[1], I. WU[2], J. C. LEITER[5], D. C. LU[2]; [1]UCLA Chapter, Los Angeles, CA; [2]UCLA, Los Angeles, CA; [3]USC, Los Angeles, CA; [4]Oakland Univ. William Beaumont Sch. of Med., Rochester, MI; [5]Dartmouth, Hanover, NH

Abstract: Phase resetting is the activity of an oscillator that restarts with interruption before it completes a cycle. In the central nervous system (CNS) of humans and other mammals, phase resetting of an oscillator often indicates the change in the firing patterns of neurons that contribute to establishing the time frame of the oscillator. Among the oscillators in the human CNS, the respiratory central pattern generator (CPG) is one of the oscillators that are essential for life. Investigating the phase resetting of respiration not only elucidates the neuronal structure but also helps to develop therapeutic interventions for respiratory dysfunction. In this study, we discovered that electrical epidural stimulation (EES) delivered at the dorsal cervical spinal cord can induce respiratory phase resetting in human subjects. Respiratory phase resetting was analyzed by noting the change in phase (or not) when the onset of EES occurred at different times of the respiratory cycle in the anesthetized human subjects with spontaneous breathing. We measured the respiratory phase shift during the spontaneous respiration and compared phase resetting between the stimulation and sham (no stimulation control) tests at seven different cervical levels. Respiratory cycle phase resetting was more common during 5 or 30 Hz EES than

during sham stimulation. Resetting may advance or delay the respiratory cycle. Therefore, we took the absolute value of the change in phase angles and transformed these absolute values using the Box-Cox transformation since angles are not normally distributed. After transformation, the two-way ANOVA and paired comparisons indicated that the EES-induced phase angle changes observed after the start of 5 Hz EES and 30 Hz EES was significantly greater than the spontaneous variation of phase angles in the sham condition ($p < 0.0001$ for both sham vs. 5 Hz and sham vs 30 Hz). Our finding is consistent with the previous studies reporting respiratory settings after single pulses of dorsal cervical epidural stimulation, but not after ventral after EES. This observation suggests that spinal EES activates elements of the supraspinal respiratory CPG via recruitment of the propriospinal neurons and afferent sensory pathways in the dorsal spine or by activating a local spinal respiratory CPG.

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Poster

P625. Respiratory Control II

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Program #/Poster #: P625.02

Topic: E.08. Respiratory Regulation

Support: Louis and Harold Price Foundation
H & H Evergreen Foundation
J. Yang Family Foundation

Title: Electrical epidural stimulation (EES) reverses the respiratory suppression induced by optoinhibition of the brainstem somatostatin neurons.

Authors: *K. LI[1], R. HUANG[2], M. MADHAVAN[1], E. GALER[1], Y. ZHOU[1], N. WOBIG[1], M. A. BANTUGAN[2], M. ABDELSHAHID[1], M. ABDELSHAHID[2], M. YAMAMOTO[2], S. HOLLY[1], C. CEPEDA[1], M. LEVINE[1], J. C. LEITER[3], D. C. LU[2];
[1]UCLA Chapter, Los Angeles, CA; [2]UCLA, Los Angeles, CA; [3]Dartmouth, Hanover, NH

Abstract: The rhythmic neural activity that produces breathing in mammals is generated by specialized neuronal networks - the respiratory central pattern generator (CPG) - located in the brainstem. However, those regions related to the respiratory pattern generation, such as the preBotinger Complex (preBotC) and rostral ventral respiratory group (rVRG), are difficult to access. The respiratory failures due to pathologies of the central nervous system, especially the brainstem, has high mortality and morbidity rate since there is no safe and effective means to

modulate respiration through the brainstem. The stimulation at the cervical spinal cord emerges to be a novel alternative in respiratory facilitation. In the spinal cord injured and non-injured animals, pharmacogenetic stimulation of cervical excitatory interneurons enhances respiratory motor function. Furthermore, epidural electrical stimulation (EES) at cervical spinal cord facilitates the respiratory frequency in non-injured mice and human subjects. Given all these promising discoveries, it is important to investigate if the stimulation at the cervical spinal cord can facilitate respiratory drive when the respiratory circuit in the brainstem is compromised. In this study, we optogenetically suppressed the activity of somatostatin (SST)-expressing neurons in the preBotC and rVRG. This was performed in SST-Cre expressing mice injected with adeno-associated virus (AAV) that harbored Cre-dependent halorhodopsin. EES was delivered between cervical level 3 and 4 at 30 hertz frequency for 30 seconds during the opto-inhibition. The thirty-second EES was sufficient to reverse the respiratory inhibition caused by optogenetics during the stimulation and the respiration perpetuated another 30 second after the EES stopped under the opto-inhibition. Our findings above suggest that the cervical spinal cord could serve as a neuromodulation portal to activate the respiratory CPG.

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Poster

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Program #/Poster #: P625.03

Topic: E.08. Respiratory Regulation

Support: BCM McNair Scholar Program
R01HL130249

Title: The role of glutamatergic signaling in central noradrenergic neurons in respiratory control

Authors: *Y. CHANG[1], R. RAY[2];
[1]Mol. Physiol. and Biophysics, [2]Deparment of Neurosci., Baylor Col. of Med., Houston, TX

Abstract: Central noradrenergic (NA) neurons are key constituents of the respiratory homeostatic network. NA dysfunction is implicated in several developmental respiratory disorders such as the fatal Sudden Infant Death Syndrome (SIDS) and Rett Syndrome, in which abnormal chemosensory function is implicated. However, which NA populations modulate respiratory chemoreflexes and the underlying mechanisms are still not fully understood. Histological evidence suggests that glutamate is co-transmitted in subsets of brainstem NA

neurons, some of which may contribute to regulating chemoreflexes. However, the extent of glutamate co-transmission in the central NA system remains uncharacterized. Further, it remains unclear what role NA-based glutamatergic signaling plays in various aspects of respiratory homeostasis. To anatomically map potential glutamate-expressing NA neurons, we characterized the expression profile of all three known of Vesicular Glutamate Transporters (Vglut1, Vglut2 and Vglut3) in NA neurons by intersectional genetic fate mapping. For each Vglut1/2/3 transporter, a corresponding Cre knock-in recombinase line was combined with *DBH-p2a_Flpo* mice. These *Vglut1/2/3_Cre; DBH-p2a_Flpo* compound lines were crossed with the *RC::F_TdTomato_P_GFP* intersectional reporter mice. In each of the three intersectional reporter crosses, NA neurons co-expressing either Vglut1, Vglut2, or Vglut3 are labeled by green fluorescent protein (GFP) while NA neurons without any Vglut1/2/3 expression are labelled by red fluorescent protein (tdTomato). Our preliminary cumulative fate maps found expression of one or more glutamate transporters in every single NA nucleus in adult mice. Importantly, these fate maps reflect the cumulative expression profiles of glutamate markers in NA neurons throughout the development and lifetime of the mouse. To determine what physiological role, if any, Vglut2-based glutamatergic signaling may play in adult respiratory homeostasis, we conditionally ablated Vglut2 in central NA neurons by crossing *DBH-Cre/+* with *Vglut2*$^{flox/flox}$ mice. Mutants and sibling controls (6-8 weeks) were assessed for respiratory function by whole-body barometric plethysmography. Ventilation and VO2 were assessed under room air (21%O_2,79%N_2), hypercapnia (21%O_2, 74%N_2, 5%CO_2) and hypoxia (10% O_2, 90% N_2). Our preliminary data indicates that loss of Vglut2 in NA neurons does not significantly impact breathing under room air, hypercapnia or hypoxia, suggesting that Vglut2-based glutamatergic signaling in the central NA system may be dispensable for several aspects of ventilatory control.

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Poster

P625. Respiratory Control II

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Topic: E.08. Respiratory Regulation

Support: NIH Grant R01HL142752 (JJW and TLB)
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Title: Butyrate supplementation rescues sex-specific deficits in respiratory plasticity and mean arterial pressure in adult offspring exposed to gestational intermittent hypoxia

Authors: *C. R. MICKELSON, E. P. KOBITTER, A. L. MEZA, A. B. RADCLIFF, K. K. HILL, R. SONG, J. S. MISHRA, S. KUMAR, J. J. WATTERS, T. L. BAKER;
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Abstract: Sleep disordered breathing (SDB) in pregnancy is increasing in parallel with the obesity epidemic, and is linked to adverse perinatal outcomes in the offspring. Consequences of maternal SDB during pregnancy on the adult offspring are not well-understood. Preliminary data indicate that adult male, but not female, offspring of dams exposed to intermittent hypoxia during gestation (GIH - gestational intermittent hypoxia), a rat model of SDB during pregnancy, have elevated mean arterial pressure (MAP), increased neuroinflammation, inflammation-induced impairment of homeostatic plasticity in the respiratory control system, and gut dysbiosis. Specifically, adult male GIH offspring have decreased relative abundance of gut bacteria that produce butyrate, a short-chain fatty acid that has potent anti-inflammatory properties and regulates immune function in the central nervous system. Since literature suggests that gut dysbiosis, neuroinflammation, and hypertension exist in a complicated vicious cycle, we treated male GIH offspring with tributyrin, a butyrate prodrug. Prior to tributyrin treatment, MAP in male GIH offspring was 96±3 mmHg, versus 76±1 mmHg in control rats receiving gestational intermittent normoxia (GNX). Similarly, male GIH offspring exhibited deficits in respiratory plasticity in response to recurrent central apneas (3.6±6.8% plasticity), when compared to GNX offspring (59.3±4.8% plasticity). GIH offspring exhibited evidence of elevated neuroinflammation in brain regions underlying respiratory control (e.g., IL-1β expression 1.6±0.2-fold higher than GNX controls (1.0±0.2)). Following three weeks of tributyrin treatment, MAP had normalized (80±2 mmHg) and the ability to express respiratory neuroplasticity was rescued (78±30%). Studies on whether tributyrin also reduces neuroinflammation are ongoing. These data indicate that gut dysbiosis may contribute to sex-specific impairments induced by maternal exposure to intermittent hypoxia during gestation that can be rescued in adulthood by supplementing with butyrate.

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Poster

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Topic: E.08. Respiratory Regulation

Support: NIH R01 HL142752
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Title: Endomorphin-2 at low concentrations increases respiratory motor burst amplitude in neonatal rat brainstem-spinal cord preparations

Authors: *M. G. GUMNIT, J. J. WATTERS, T. L. BAKER, S. M. JOHNSON;
Univ. of Wisconsin-Madison, Madison, WI

Abstract: Mu-opioid receptors (mOR) and their endogenous ligands are part of an intricate peptidergic neuromodulatory system that regulates respiratory neural activity. The association of opioid use and respiratory depression is well established, but the endogenous role of mOR in respiratory motor control is poorly understood. To explore this question, we tested very low (nanomolar) concentrations of mOR agonists using isolated neonatal rat (P1-P3) brainstem-spinal cord preparations and quantified respiratory-related motor output from spinal C4-C5 ventral rootlets. When DAMGO (synthetic opioid mOR agonist) or endomorphin-2 (endogenous ligand for mOR) were bath-applied for 15 min at 10-100 nM, respiratory-related motor burst frequency decreased, but there was also a large increase in burst amplitude. Burst amplitude facilitated 45 ± 38% and 32 ± 24% (mean ± stdev) above baseline for 50 nM DAMGO (n=8) and 100 nM endomorphin-2 (n=10), respectively ($p<0.001$). When DAMGO or endomorphin-2 were bath-applied at 20 nM, burst amplitude increased by 26-29% ($p<0.05$), burst frequency decreased by 10-20% ($p<0.01$), but the overall minute activity (total motor burst area per min) was unchanged at -8 ± 30% ($p=0.84$) and 1 ± 32% ($p=.84$) of baseline, respectively. These data suggest that mOR activation with low agonist concentrations transforms the respiratory network to produce a different motor pattern without compromising blood-gas homeostasis. We speculate that the endogenous mOR system in the brainstem may play a role in respiratory motor behaviors requiring a large tidal volume and low frequency, such as coughing or gasping.

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Poster

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Program #/Poster #: P625.06

Topic: E.08. Respiratory Regulation

Support: FAPESP (#2019/13249-3)
NIH (R01 HL128970, R01 HL133100, and R01 HL138932)

Title: Activation of leptin receptor positive neurons in dorsomedial hypothalamus stimulates breathing in obese mice

Authors: *M. R. AMORIM[1], X. WANG[2], H. PHO[1], S. BEVANS-FONTI[1], F. ANOKYE-DANSO[1], J. ESCOBAR[2], O. DERGACHEVA[2], L. G. S. BRANCO[3], D. MENDELOWITZ[2], V. Y. POLOTSKY[1];
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Abstract: Introduction: Obesity leads to obstructive sleep apnea (OSA), characterized by loss of upper airway muscle tone during sleep leading to carbon dioxide retention and intermittent hypoxemia. OSA leads to high neurocognitive, cardiovascular, and oncologic morbidity and mortality. There is no effective pharmacotherapy for OSA. We have previously shown that an adipose-produce hormone leptin acts in the brain to abolish hypoventilation and upper airway obstruction during sleep, and, therefore may be a promising therapeutic in obese patients with OSA. Our previous data also suggest that leptin stimulates breathing acting on the leptin receptor ($LEPR^b$) positive neurons in the dorsomedial hypothalamus (DMH), but not in the medulla. **Aim:** In this study, we examined the contribution of $LEPR^b$ positive neurons in the DMH to control of breathing and upper airway function during sleep in diet-induced obese mice. **Methods:** We expressed designer receptors exclusively activated by designer drugs (DREADD) selectively in the $LEPR^b$ positive neurons of the DMH of *Lepr^b*-Cre-GFP mice with diet-induced obesity (DIO) and examined the effect of DREADD ligand, J60, on breathing during sleep. **Results:** Chemogenetic activation of $LEPR^b$ positive DMH neurons increased inspiratory flow, respiratory rate, and minute ventilation during REM sleep, without significant changes in sleep architecture. The effect was particularly pronounced during inspiratory flow limitation, which suggest that upper airway obstruction during REM sleep was relieved. Activation of $LEPR^b$ positive DMH neurons with a DREADD ligand J60 increased the hypercapnic ventilatory response (HCVR) and this response was blunted by a serotonin receptor antagonist, methysergide. *In vitro* experiments revealed that DMH $LEPR^b$ neurons project to the serotonergic neurons in the dorsal raphe and that optogenetic stimulation of DMH $LEPR^b$ neurons evoked excitatory post-synaptic currents in ChR2 expressing neurons. **Conclusion:** Leptin up-regulates the hypercapnic ventilatory response and upper airway patency during sleep acting on $LEPR^b$ neurons in the DMH. Respiratory effects of leptin are likely mediated via projections of these neurons to the dorsal raphe.

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Poster

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Topic: E.08. Respiratory Regulation

Support: CIHR

Title: Glutamatergic preBötzinger Complex neurons as potential targets to alleviate opioid-induced respiratory depression

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Abstract: The current opioid epidemic in North America is staggering and has been exacerbated by the COVID19 pandemic. Opioid drugs are highly addictive, and when abused and overdosed can lead to lethal respiratory depression. The preBötzinger Complex (preBötC) is a small brainstem region generating respiratory rhythm and mediating opioid-induced respiratory depression (OIRD). In the preBötC, a large population of neurons are glutamatergic and co-express μ-opioid receptors (MOR). Blocking MOR in the preBötC reverses OIRD, suggesting that preBötC neurons may be potential targets to selectively prevent respiratory depression. However, it is unknown whether glutamatergic neurons may serve as druggable targets for the development of an antidote for OIRD. To specifically stimulate glutamatergic preBötC neurons, we expressed the Designer Receptors Exclusively Activated by Designer Drugs, hM3Dq (an engineered version of the M3 muscarinic receptor), in neurons with vesicular glutamate transporter 2 (VGLUT2, a marker of glutamatergic neurons) using a cre-lox recombination approach. We unilaterally injected the viral vector (AAV-hM3Dq-mCherry) in the preBötC region of Vglut2-IRES-Cre male mice (Jackson Labs stock # 016963). Four weeks after virus injection, mice were anesthetized, and electrodes were implanted in the diaphragm and genioglossus muscles to measure respiratory activity. OIRD was induced by injection of fentanyl (5 μg/kg) and VGLUT2 cells were activated with the hM3Dq ligand clozapine-N-oxide (CNO, 1 mg/kg). Mouse brains were collected to quantify hM3Dq expression in the brainstem. Preliminary data show that activation of VGLUT2 preBötC neurons with CNO increased respiratory rate. This suggests that it would prevent respiratory rate depression by fentanyl when compared to mice receiving fentanyl alone. Further experiments aim to demonstrate whether hM3Dq-mCherry is specifically expressed in preBötC cells and co-expressed with MORs. OIRD will also be assessed in freely-behaving mice using whole-body plethysmography. Preliminary data suggest that glutamatergic preBötC neurons may be considered as potential targets for the development of preventive drug therapies for respiratory depression during opioid overdose.

Disclosures: N. Parzei: None. **C. Scarpellini:** None. **G. Montandon:** None.

Poster

P625. Respiratory Control II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P625.08

Topic: E.08. Respiratory Regulation

Support: NIH Grant R01 HL148030
NIH Grant T32 HL134621

Title: Spinal Adenosine 2A receptor inhibition amplifies moderate acute intermittent hypoxia-induced phrenic long-term facilitation in aging male rats

Authors: *A. B. MARCIANTE, G. S. MITCHELL;
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Abstract: Phrenic long-term facilitation (pLTF) is a form of respiratory plasticity elicited by acute intermittent hypoxia (AIH). AIH activates competing cellular mechanisms of phrenic motor plasticity, initiated by serotonin 2 and adenosine 2A (A_{2A}) receptors on phrenic motor neurons, respectively. With moderate AIH (mAIH), the serotonin-dependent Q pathway to phrenic motor facilitation dominates, with an adenosine constraint. Consequently, spinal A_{2A} receptor inhibition blocks this adenosinergic constraint and enhances mAIH-induced pLTF. With increasing age, pLTF decreases due to progressively declining levels of circulating testosterone and glial-derived estrogen levels in males and post-menopausal females in the CNS. We hypothesize that, in aged male rats, mAIH triggers greater glial adenosine release, increasing the "brake" on the still dominant Q pathway to phrenic motor facilitation, thereby decreasing pLTF. Thus, we predict that blocking spinal A_{2A} receptors in aged male rats will release the age-enhanced adenosine constraint, restoring pLTF to levels equal to or above those observed in young male rats. To test this hypothesis, a selective A_{2A} receptor antagonist (MSX-3; 10 uM, 12 uL) or vehicle (0.9% sterile saline) were delivered intrathecally (C4) in anesthetized, vagotomized, paralyzed and ventilated young (3-6 months) and aged (20-22 months) male Sprague-Dawley rats prior to mAIH (3, 5 min hypoxic episodes; arterial Po_2 = 45-55 mmHg). In young males, mAIH consistently and reproducibly induces pLTF of approximately 60% above baseline at 60 minutes post-hypoxia. As expected, A_{2A} receptor inhibition significantly enhanced mAIH-induced pLTF in young male rats (n = 6; 118.5 $\pm$ 5.4%) versus vehicle controls (n = 6; 55.3 $\pm$ 8.7%; $p < 0.001$) and time controls (n = 6; 11.7 $\pm$ 4.4%; $p < 0.001$). In aged males, A_{2A} receptor inhibition enhanced mAIH-induced pLTF to an even greater extent (n = 6; 180.3 $\pm$ 21.3%) versus vehicle controls (n = 4; 29.4 $\pm$ 19.4%; $p < 0.001$) and time controls (n = 3; 2.3 $\pm$ 6.8%; $p < 0.001$). Preliminary data suggest aged female rats (16-18 months) also have enhanced mAIH-induced pLTF with A_{2A} receptor inhibition (n = 2; 148.8 $\pm$ 1.9%) versus vehicle controls (n = 2; 46.2 $\pm$ 3.7%) and time controls (n = 2; 5.3 $\pm$ 17.2%) in estrus. These findings advance our understanding of age-related sexual dimorphism in intermittent hypoxia induced phrenic motor plasticity. Understanding age and sex effects on AIH-induced respiratory motor plasticity is fundamental as we translate and develop new therapies for severe neuromuscular disorders that compromise breathing and other movements.

Disclosures: A.B. Marciante: None. **G.S. Mitchell:** None.

Poster

P625. Respiratory Control II

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Program #/Poster #: P625.09

Topic: E.08. Respiratory Regulation

Title: Suppression of apneas by dextromethorphan in Sprague Dawley rats

Authors: *M. W. CALIK;
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Abstract: Obstructive sleep apnea (OSA) prevalence in American men and women is estimated at 14% and 5%, respectively, and prevalence rates are increasing. OSA's acute physiological effects (hypoxemia/hypercapnia, fragmented sleep, etc.) can develop into long-term sequelae (diabetes, cardiovascular morbidities, etc.). There are no pharmacological treatments for OSA. Accumulating evidence indicates the potential importance of afferent vagus nerve activity and its central synaptic targets in modifying respiratory control. Recent work has shown that cannabimimetic treatment using dronabinol (Dron) in rats decreased sleep apnea duration/events through activation of cannabinoid receptors on nodose ganglia; similar cannabimimetic decrease in apneas has been shown in humans. However, cannabinoids have also been shown to decrease REM sleep in rats and humans. Though it is the most advanced candidate for a pharmacotherapy for OSA, Dron is a controlled substance with substantial psychogenic effects. Thus, further research is needed to identify better pharmacological treatments. Dextromethorphan (DEX), a safe nonprescription antitussive, has been shown to inhibit serotonin-induced inward current in rat nodose ganglion cells. However, it is unknown if DEX can decrease apnea propensity. Here, we hypothesized that DEX would decrease apneas in rats. Two experiments were conducted. In the first experiment, using an established animal model of reflex apnea, adult male Sprague Dawley rats were anesthetized and instrumented with bilateral electrodes to monitor genioglossi EMG and with a piezoelectric strain gauge to monitor respiratory pattern. Serotonin was IV infused to induce apnea. DEX was injected into each nodose ganglion and IV serotonin infusion was repeated to induce apneas. In the second experiment, using a conscious animal model of sleep, adult male Sprague Dawley rats were individually placed in PiezoSleep cages to noninvasively monitor breathing and sleep. Before placement into the cages, a repeated measures random order crossover design was implemented, such that each rat received each of 4 IP injections exactly one time: vehicle alone (DMSO); Dron alone; DEX alone; or Dron/DEX combination. Our results show that DEX injected into the nodose ganglia decreased reflex apneas and increased upper airway activation compared to controls. In conscious rats, IP DEX showed no effect on sleep efficiency; Dron, however, decreased sleep efficiency as previously. IP DEX and IP Dron decreased apneas, and combination of Dron/DEX had the largest decrease in apneas. Collectively, the results show the feasibility of DEX as a potential pharmacological treatment for OSA.

Disclosures: M.W. Calik: F. Consulting Fees (e.g., advisory boards); Signal Solutions, Inc..

Poster

P625. Respiratory Control II

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Program #/Poster #: P625.10

Topic: E.08. Respiratory Regulation

Support: NIH P01 HL090554
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NIH R01 HL126523
Fapesp #2019/11969-2

Title: The optogenetic stimulation of PiCo neurons increases the sympathetic activity during postinspiration in vivo mice

Authors: *M. KARLEN-AMARANTE[1,2], A. D. HUFF[1], N. A. BAERTSCH[1], D. S. A. COLOMBARI[2], J. RAMIREZ[1,3];
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Abstract: Inspiratory rhythmic activity is generated by the synchronized activity of the preBötzinger complex (preBötC), a well-known heterogenous network consisting of glutamatergic and glycinergic/GABAergic neurons that are essential for breathing. Recent studies identified the so called postinspiratory complex (PiCo), a rhythmogenic network which is critical for generating postinspiratory (post-I) activity in vivo and in vitro. Like the preBötC, PiCo contains inhibitory and excitatory neurons. The excitatory neurons are characterized by a cholinergic/glutamatergic transmitter phenotype. There is increasing evidence that respiratory neurons also interact with presympathetic neurons involved in the generation of sympathetic activity. This interaction has been attributed to the increased sympathetic activity and changes in respiratory-sympathetic coupling following exposure to chronic intermittent hypoxia (CIH). To investigate the contribution of PICo in the respiratory-sympathetic coupling, we used an optogenetic *in vivo* mouse preparation previously exposed to CIH for 3 weeks (5% O_2 - 80 bouts/day). The ventral surface of the brainstem in spontaneously breathing, anaesthetized (Urethane 1.5mg/kg) CHATCre/Ai32 adult and Vglut2cre/Ai32 mice was exposed and an optical fiber positioned bilaterally over the PiCo, while recording the diaphragm muscle (DIA), hypoglossal (HN), cervical vagus (cVN) and cervical sympathetic nerves (cSN). Our data show that CIH exposure increased the respiratory frequency, post-I vagus activity and sympathetic activity during all respiratory phases. The predominance of inspiratory coupled sympathetic activity seen in controls was reduced after CIH exposure due to an increased sympathetic activation during the post-I and late expiratory (E2) activity phase. Random optogenetic

stimulation (200ms) of PiCo Chat or Vglut neurons (~ 9mV, 447nm) in control mice significantly promoted sympathetic activity during post-I activity, thereby blunting the difference between post-I and E2. We conclude that CIH increases baseline respiratory frequency and sympathetic activity *in vivo* and suggests that an increased PICo activation may contribute to increased sympathetic drive during post-I and hypertension in CIH.

Disclosures: M. Karlen-Amarante: None. **A.D. Huff:** None. **N.A. Baertsch:** None. **D.S.A. Colombari:** None. **J. Ramirez:** None.

Poster

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Program #/Poster #: P625.11

Topic: E.08. Respiratory Regulation

Support: NIH HL111215
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Commonwealth of Kentucky Challenge for Excellence
Craig F. Neilsen Foundation CNF546714

Title: Impact of the laryngeal adductor reflex on swallow-breathing relationship during high respiratory drive

Authors: *K. YOUNG, K. E. ICEMAN, T. PITTS;
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Abstract: Authors: Kellyanna Young[1], Kim Iceman[1], Teresa Pitts[1]
Author Affiliations[1]Department of Neurological Surgery and Kentucky Spinal Cord Injury Research Center, College of Medicine, University of Louisville, Louisville, KY, United States of America
Title: Impact of the laryngeal adductor reflex on swallow-breathing relationship during high respiratory drive
Swallow is initiated by oral/pharyngeal afferent feedback, but it must coordinate with breathing. This relationship is critical for maintaining a clear airway and reducing risk of aspiration. Swallow is modulated by afferent input, and numerous other reflexes also act to protect the airway and must coordinate with swallow and breathing. The laryngeal adductor reflex (LAR) is a rapid reflex which closes the glottis and limits the chance of aspiration. We induced LAR and swallowing in anesthetized spontaneously breathing cats by infusing water into the oropharynx under conditions of room air or 10% CO_2. Electromyogram activity was measured using bipolar electrodes inserted into the upper airway, chest wall, and diaphragm muscles. In cats breathing

room air, 90% of swallows occurred during expiration (E), 10% during the transition from inspiration to expiration (I-E), and 0% during inspiration (I). Under hypercapnic conditions of 10% CO2, swallows occurred more frequently during I (31%), 19% during I-E transition, 22% during E-I transition, and 28% during E. On room air, 100% of the LARs occurred during E. During hypercapnia, LARs occurred during I (13%), I-E transition (25%) and E-I transition (63%). Overall, hypercapnia significantly reduced LAR occurrence. These results suggest that the LAR is suppressed during high respiratory drive, which causes swallow to occur more frequently during inspiration. This confluence of factors may increase risk of aspiration during periods of high respiratory drive. Research was supported by the NIH HL111215 and NS110169; the Kentucky Spinal Cord and Head Injury Research Trust; the Commonwealth of Kentucky Challenge for Excellence; and the Craig F. Neilsen Foundation CNF546714.

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Poster

P679. Neural Bases of Impulsivity and Risky Behavior

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P679.01

Topic: H.03. Decision Making

Support: CIHR Project grant to CAW (PJT-162312)
UBC Psychiatry salary support to TJH

Title: Accumbal acetylcholine is a critical modulator of cue-induced risk-taking

Authors: *C. WINSTANLEY[1], L. CALDERHEAD[2], A. LI[2], G. D. BETTS[3], C. CHERNOFF[2], S. RAMAIAH[2], K. HRELJA[2], T. HYNES[2];
[1]Univ. British Columbia, Vancouver, BC, Canada; [2]Univ. of British Columbia, Vancouver, BC, Canada; [3]UBC, Vancouver, BC, Canada

Abstract: The intense audiovisual stimuli of modern casinos may promote riskier play - a phenomenon that can be modeled preclinically using the rat gambling task. Rats are given a choice between large, unlikely rewards (risky) and smaller, more certain ones (optimal). When casino-like cues are introduced, the proportion of risk-preferring rats is enriched over time. We theorize that the phasic dopamine release evoked by these cues causes compensatory changes in the dopamine system to promote risk-taking. The neurons supplying acetylcholine to nucleus accumbens core (NAc) are powerful titrators of NAc dopamine release. Via local terminal modulation of dopamine neurons, NAc acetylcholine contributes to the maintenance of tonic NAc dopamine. Motivationally relevant cues halt NAc acetylcholine release, thus stopping the tonic stream of dopamine, giving way to the influence of the cue-evoked phasic dopamine we theorize to potentiate risk-taking. We therefore sought to leverage the NAc acetylcholine system

to manipulate cue-driven risk taking. By depleting NAc acetylcholine and consequently reducing tonic DA release, we hypothesized that the cue-induced phasic DA signal would have a greater impact (i.e., greater signal to noise ratio), thereby enhancing cue-induced risk-taking. Driving NAc acetylcholine release should, conversely, decrease the signal-to-noise ratio of cue-induced tonic DA release and foster an optimal strategy. Using DREADDs in combination with female and male ChAT:cre transgenic rats, we bidirectionally modulated the cholinergic neurons supplying the NAc. In separate cohorts, we performed the manipulation chronically as rats learned the task and acutely in well-trained rats. In rats that underwent chronic inhibition during learning, a longitudinal increase in risk taking was observed, while excitation resulted in a reduction. Interestingly, the inhibition effect was observed only in females and the excitation effect only in males -- this may be due to baseline differences in tonic dopamine levels across the sexes. Acutely inhibiting NAc acetylcholine had a less pronounced effect, supporting our theory of a cue-induced longitudinal emergence of a risk-taking phenotype. These findings furthermore point to biological sex as an important consideration in the development of pharmacotherapies.

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Poster

P679. Neural Bases of Impulsivity and Risky Behavior

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Program #/Poster #: P679.02

Topic: H.03. Decision Making

Support: CIHR Project grant to CAW (PJT-162312)
UBC Psychiatry salary support to TJH

Title: Midbrain dopamine neurons are powerful effectors of addiction-like behaviour

Authors: *T. HYNES[1], K. HRELJA[1], C. CHERNOFF[1], M. TSE[3], G. D. BETTS[1], M. LYSENKO-MARTIN[5], B. RUSSELL[1], B. RUSSELL[1], L. CALDERHEAD[1], A. LI[1], S. KAUR[2], S. B. FLORESCO[4], C. A. WINSTANLEY[3];
[2]Psychology, [1]Univ. of British Columbia, Vancouver, BC, Canada; [3]Psychology, [4]Univ. British Columbia, Vancouver, BC, Canada; [5]McMaster Univ., Hamilton, ON, Canada

Abstract: Addiction is a complex affliction, characterized not only by the use of drugs, but also by deficits in cost/benefit decision making. A critical biological factor in the development of addiction is aberrant dopamine (DA) neurotransmission. Biological sex is also an important consideration. Investigating such phenomena is difficult in humans, because of the profound influence the socio-political construct of gender has on behaviour. We therefore sought to longitudinally probe the complex intersection of addiction, decision making, sex, and DA

neurotransmission by combining our cued rat gambling task (crGT; a rodent assay of cost/benefit decision making) with operant intravenous cocaine self-administration and chemogenetic manipulations of DA neurons. We infected the midbrain DA neurons of female and male rats with either excitatory or inhibitory designer receptors, before training them in our crGT. Prior to daily crGT training sessions, we administered clozapine-N-oxide to activate the receptor and modulate the excitability of midbrain DA neurons. Once rats achieved stable crGT performance, we allowed them to self-administer intravenous cocaine. In females, both inhibition and excitation of midbrain DA neurons increased risk-taking. Excitation of midbrain DA neurons also increased impulsivity and psychomotor sensitization to cocaine. In males, risk-taking was reduced by inhibition of midbrain DA neurons and their projections to the accumbens. Inhibition of midbrain DA neurons furthermore decreased impulsivity in males. In both sexes, inhibition or excitation of midbrain DA neurons increased cocaine-taking and psychomotor sensitization to cocaine but prevented cocaine-induced deficits in decision making. Regardless of direction, upsetting the balance of the DA system has generally deleterious effects in females. For males, dopaminergic inhibition is generally protective against addiction-relevant behaviours, while excitation of the system is pro-addictive. Excitation was furthermore associated with sensitized phenotype (i.e., increased phasic activity of DA neurons locomotor reactivity to cocaine). These findings shed light on the still outstanding question as to how both hyper- and hypo-dopaminergic states contribute to addiction vulnerability.

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Poster

P679. Neural Bases of Impulsivity and Risky Behavior

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Program #/Poster #: P679.03

Topic: H.03. Decision Making

Support: CIHR Grant PJT-162312

Title: In order to drive risky choice, cues must be predictable and win-paired: a computational modelling analysis

Authors: *D. R. KIM, S. B. A. MALHAS, C. A. WINSTANLEY, B. A. HATHAWAY; Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Win-associated cues can enhance disadvantageous risky choice in both rats and humans, and the impact of cues on risky choice is a major component of many theories of addiction. However, the role of specific elements of such cues in producing this behavioural

effect has not been thoroughly investigated. Accordingly, the present experiments assessed the effect of five different cue administration paradigms on reinforcement learning and risky decision making.
Four cohorts of 32 to 64 male Long-Evans rats were trained on six versions of the rat Gambling Task (rGT), a rodent analogue of the human Iowa Gambling Task. This task was designed such that the optimal strategy for earning sugar pellets over time is to favour options paired with lower per-trial gains, as these are associated with a higher probability of winning and shorter time-out penalties. Consistently selecting the high-risk, high-reward options - which feature larger per-trial gains but longer and more frequent time-out penalties - leads to less reward overall. Audiovisual cues were paired with differing sets of outcomes to create five variants of the rGT. On the standard-cued version of the task, audiovisual cues are delivered only on winning trials, and scale in magnitude and complexity with reward size. For the reverse-cued task, the cues are reversed such that the longest and most complex cue is paired with the smallest reward. For the outcome-cued task, cues are delivered with every outcome - both wins and losses. For the random-cued task, cues are delivered with a 50% probability across both winning and losing trials. Finally, the loss-cued task only delivers cues on losing trials.
As observed in previous experiments, there was a higher proportion of risk-preferring rats on the standard-cued task compared to the uncued task. The outcome-cued and reverse-cued task variants produced a similar risky choice profile to the standard-cued task. Conversely, rats trained on the loss-cued and random-cued variants did not exhibit this cue-induced risk preference. Early computational analyses indicate that win-paired cues reduce the impact of losses on decision making, and the impact of wins is increased when cues track reward size. These results suggest that cues do not increase risky choice by simply elevating arousal, or amplifying the difference between wins and losses. Instead, it is important that the cues are associated with wins, and that they occur on every win. Interestingly, if such conditions are met, presenting the cues on losing outcomes does not diminish their ability to drive risky choice. These results may help us understand how sensory stimulation can increase the addictive nature of gambling products.

Disclosures: D.R. Kim: None. **S.B.A. Malhas:** None. **C.A. Winstanley:** None. **B.A. Hathaway:** None.

Poster

P679. Neural Bases of Impulsivity and Risky Behavior

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Program #/Poster #: P679.04

Topic: H.03. Decision Making

Support: CIHR project grant (PJT-162312)

Title: The effects of selective activation of the dopamine D2 and D3 receptors on risk preference in male rats

Authors: *B. RUSSELL[1], H. BRODIE[1], K. HRELJA[1], S. ANSARY[1], T. CHANG[1], C. A. WINSTANLEY[2];
[1]Univ. of British Columbia, Vancouver, BC, Canada; [2]Psychology, Univ. British Columbia, Vancouver, BC, Canada

Abstract: Dopamine D2/D3 receptor agonists, like ropinirole, are commonly prescribed to individuals with Parkinson's disease. Although these drugs are effective at alleviating Parkinsonian motor symptoms, they have also been found to induce gambling disorder and other behavioural addictions, as well as impulse control disorders, in a substantial minority of patients. However, the underlying mechanism by which these drugs induce these behaviours has been debated. There are findings to support the role of both the D2 receptor and D3 receptor in the development of iatrogenic gambling disorder, with some studies suggesting that activation of the D3 receptor, and not the D2 receptor, is precipitating these disorders. Previous research has shown that the administration of non-selective dopamine D2/D3 receptor agonists can increase preference for uncertainty and risky decision-making in healthy male rats. Thus, the purpose of the current experiment was to examine whether the D2 receptor and/or the D3 receptor can independently induce risky decision-making in male rats on the cued rat gambling task (cued rGT), an adaptation of the Iowa Gambling Task (IGT). 88 male Long Evans rats were trained on the cued rGT, in which rats choose between four options associated with distinct magnitudes and probabilities of reward (1-4 sugar pellets) or time-out penalties (5-40s). The optimal strategy is to favour options associated with smaller per trial gains, but shorter penalties. Following training to nose-poke for reward, rats received daily injections of the selective D2 agonist sumanirole (n=16 at 0.10 mg/kg and n=16 at 0.65 mg/kg), the selective D3 agonist PD128907 (n=16 at 0.10 mg/kg and n=16 at 0.60 mg/kg), or saline (n=24) prior to testing. These injections were administered throughout the acquisition phase of the cued rGT and until behavioural stability on the task was reached. Next, the rats completed 8 sessions on the cued rGT without any drug being administered prior to testing. Although 0.10 mg/kg of PD128907 increases risky choice when given acutely once rats have acquired the task, chronic administration during acquisition at this dose did not increase risk preference. The results from this experiment will offer insight into the selective contribution of D2 and D3 receptor agonism on decision making and the development of both iatrogenic and idiopathic gambling disorder and other addictive disorders.

Disclosures: B. Russell: None. **H. Brodie:** None. **K. Hrelja:** None. **S. Ansary:** None. **T. Chang:** None. **C.A. Winstanley:** None.

Poster

P679. Neural Bases of Impulsivity and Risky Behavior

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Program #/Poster #: P679.05

Topic: H.03. Decision Making

Support: CIHR Project Grant: PJT 162312

Title: Excitation of substantia nigra pars compacta increases motor impulsivity during probabilistic decision making

Authors: *G. D. BETTS, B. A. HATHAWAY, T. J. HYNES, C. A. WINSTANLEY;
Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Dopamine has a significant influence on decision making and impulsivity - two defining phenotypes of many neuropsychiatric disorders. A critical node of DA neurotransmission is the substantia nigra pars compacta (SNc), which makes it a promising target for pharmacotherapy. The necessary experimental manipulations to explore the behavioral role of SNc dopamine in humans, however, is prohibitive. We therefore sought to determine the role of SNc dopamine neurons in the cued rat gambling task (rGT) - a translationally-valid model of impulsivity and decision making. We transfused the SNc of TH::Cre transgenic rats with a DIO viral vector carrying the construct for the DREADD hM3D(Gq), allowing us to gain excitatory control over the DA neurons projecting from the region. Prior to each cued rGT training session, we administered clozapine-N-oxide (1.0 mg/kg for 30 sessions) to actuate the DREADD for the duration of the task. We found that Gq stimulation of SNc dopamine neurons increased impulsivity, while having no effect on decision making. This impulsivity could not be attributed to hyperlocomotion, as Gq stimulation had a small effect of increasing response latencies. Further, this increase of impulsivity and response latency was present only in risk-preferring rats. These findings are unique from Gq stimulation of the ventral tegmental area dopamine neurons, which causes deficits in decision making while leaving impulsivity intact. The SNc preferentially innervates the dorsal striatum, whereas the ventral tegmental area projects more ventrally; albeit a simplified interpretation, it is possible that the present discovery points to dissociable striatal loci that govern dopaminergic control over decision making (ventral tegmental area) as opposed to impulsivity (SNc). Projection-specific manipulations of SNc dopamine neurons will be conducted to further investigate this theory.

Disclosures: G.D. Betts: None. **B.A. Hathaway:** None. **T.J. Hynes:** None. **C.A. Winstanley:** None.

Poster

P679. Neural Bases of Impulsivity and Risky Behavior

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P679.06

Topic: H.03. Decision Making

Title: The effect of dopamine D2 receptor agonists and antagonists on decision making in a rodent cognitive effort task

Authors: *L. CALDERHEAD, C. WINSTANLEY;
Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Maladaptive decision-making involving effort is common in multiple psychiatric disorders. Animal models of effort-based decision making have implicated mesolimbic dopamine in regulating motivation to engage in effortful tasks to obtain greater rewards. While this effort can be physical or cognitive, these two forms have overlapping but distinct neural circuitry. A rodent cognitive effort task (rCET) has previously been developed to study such decision making in rats. The psychostimulant amphetamine has been shown to influence decision making on the rCET. When administered amphetamine rats who previously preferred low effort options (slackers) to choose high effort options more and those who preferred high effort (workers) to choose the low effort option more. However, the biological mechanism of amphetamines action is unknown. Here we conduct confirmatory research to examine the effect of pharmacological dopamine D2 receptor agonism and antagonism on male Long-Evans rats choice behaviour on the rCET while replicating previous amphetamine results and determining if the effects of amphetamine can be blocked by co-administration of a D2 antagonist. The D2 antagonist L-741,626 showed no effect on choice behaviour while the D2 agonist sumanirole decreased choice of the high effort option for both worker and slacker animals. We replicated previous results of amphetamines ability to cause slackers to choose the high effort option more and workers to choose the high effort option less. Co-administration of amphetamine and L-741,626 did not alter behaviour when compared to administration of amphetamine and drug vehicle. In conclusion, we show that D2 receptor agonism plays a role in guiding effortful decision making however its action is not required for amphetamines effects.

Disclosures: L. Calderhead: None. **C. Winstanley:** None.

Poster

P679. Neural Bases of Impulsivity and Risky Behavior

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Program #/Poster #: P679.07

Topic: H.03. Decision Making

Support: CIHR Project grant to CAW (PJT-162312)

Title: The impact of noradrenaline on cue-driven decision making and impulsivity is dissociable across frontal brain regions

Authors: *C. S. CHERNOFF[1], J. D. SCHUMACHER[2], S. RAMAIAH[1], T. J. HYNES[1], L. CALDERHEAD[1], S. B. FLORESCO[2], C. A. WINSTANLEY[3];
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Abstract: The intense audiovisual stimuli of modern casinos are thought to contribute to the development of disorder gambling. Indeed, in both human and rat models of gambling behaviour, such casino-like stimuli can elicit cravings to gamble and promote risky decision making. These cues may act to elicit the maladaptive attentional states associated with problem gambling (e.g., 'dark flow'). Critically, noradrenaline underlies the neurobiology associated with attending to salient environmental stimuli, and moreover, is known to play a role in cognitive elements of gambling (i.e., impulsivity and decision making). Prefrontal brain regions, such as the orbitofrontal cortex (OFC) and the medial prefrontal cortex (mPFC) are highly implicated in such cognitive processes and receive dense noradrenergic innervation. We recently demonstrated that systemic delivery of atomoxetine, a noradrenaline transporter inhibitor, and guanfacine, an α_2-adrenergic receptor agonist, reduce risk preference and motor impulsivity in the cued rat gambling task (crGT, a rodent cost/benefit decision making paradigm). We now seek to determine which frontal regions mediate the effects of these noradrenergic drugs. Guide cannulae were implanted in the lateral OFC (lOFC, n=15) or prelimbic cortex (PrL; n=16) of male rats trained on the crGT. Each rat received a high and low dose of both guanfacine and atomoxetine. Only atomoxetine improved decision making on the crGT when infused into the lOFC. Neither drug influenced motor impulsivity when delivered into the lOFC, yet intra-PrL administration of both atomoxetine and guanfacine reduced impulsive premature responses. Our findings here substantiate the role of prefrontal noradrenaline in cue-mediated decision making and impulsivity, and highlight the different brain regions that may guide these separate but related behaviours.

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Poster

P679. Neural Bases of Impulsivity and Risky Behavior

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P679.08

Topic: H.03. Decision Making

Support: CIHR Grant PJT-162312

Title: Modulating dopaminergic nigrostriatal activity can accelerate or delay the development of risky choice patterns driven by reward-paired cues in female rats

Authors: *B. A. HATHAWAY, A. C. LI, H. G. BRODIE, C. A. WINSTANLEY;
Univ. of British Columbia, Vancouver, BC, Canada

Abstract: It is well-established that risky decision making can play a critical role in the development and maintenance of addiction. Reward-concurrent cues may mediate this relationship, as previous results have indicated that such stimuli enhance risk preference and may potentiate cocaine intake in rats. Despite extensive addiction research implicating the dorsal striatum in compulsive drug seeking, the role that dorsal striatal dopaminergic activity plays in the development of risk preference driven by reward-paired cues remains unclear.
Accordingly, the present studies examined the effects of chronic inhibition or activation of the dopaminergic nigrostriatal pathway during the learning phase of a risky decision-making task in rats. Female TH:Cre rats (n = 32) and transgene negative litter mates (n = 32) were trained on the cued version of the rat gambling task, a rodent analogue of the human Iowa Gambling Task, to assess cue-enhanced risky decision making. This task was designed such that the optimal strategy for earning sugar pellets over time is to favor options paired with lower per-trial gains, as these are associated with a higher probability of winning and shorter time-out penalties. Consistently selecting the high-risk, high-reward options ultimately results in longer and more frequent time-out penalties, and therefore less reward overall. Adding reward-paired audiovisual cues to the task results in a higher proportion of rats that prefer the disadvantageous, risky options at baseline.
To assess nigrostriatal involvement in the development of choice preferences on the cued rGT (cRGT), Cre-dependent inhibitory hM4D(Gi) DREADD or excitatory hM3D(Gq) DREADD was infused into the substantia nigra. Rats received 1 mg/kg injections of Clozapine-N-Oxide prior to task training for the first 30 sessions of the cRGT. Risk-preferring rats were uniquely affected by these manipulations. Chronic activation of this pathway during task learning delayed the establishment of a risky decision-making profile in risk-preferring rats, whereas inhibition accelerated the development of their preference for the risky options. Motor impulsivity was bidirectionally modulated in all rats, indicated by an increase in premature responding with nigrostriatal activation and decreased premature responding with inhibition. These results provide evidence for the involvement of the dopaminergic nigrostriatal pathway in the development of cue-driven risk preference and impulsive action, therefore shedding light on its role in risky decision making associated with addiction in humans.

Disclosures: B.A. Hathaway: None. **A.C. Li:** None. **H.G. Brodie:** None. **C.A. Winstanley:** None.

Poster

P679. Neural Bases of Impulsivity and Risky Behavior

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Program #/Poster #: P679.09

Topic: H.03. Decision Making

Support: CIHR Grant F18-03649

Title: Examining the interaction between gambling, drug-taking, and decision-making using a novel behavioural task in female rats

Authors: *K. HRELJA[1], S. ANSARY[1], E. CHONG[1], B. RUSSELL[1], C. A. WINSTANLEY[2]; [1]Univ. of British Columbia, Vancouver, BC, Canada; [2]Psychology, Univ. British Columbia, Vancouver, BC, Canada

Abstract: Impaired decision-making is recognized as a diagnostic criterion for many psychiatric disorders including depression, gambling disorder, and substance use disorders. The cued rat gambling task (rGT) is translationally valuable, as it incorporates salient audiovisual cues and complex schedules of reinforcement to closely model human gambling, and accounts for factors like cost-benefit calculations and uncertainty of outcomes. In this experiment, we use a novel behavioural task to gain insight into the individual differences that exist in complex decision-making, and how volitional drug-taking impacts these cognitive processes. Female Long Evans rats (N=32) were first trained to nose-poke and lever press for reward (sugar pellet). Animals were then taught rGT contingencies and to associate one lever with the cued rGT, and the other with the uncued rGT, by enforcing forced-choice before rats could proceed to the free-choice version of the choice rGT. On the choice rGT, rats can choose which task and probabilistic outcome they want to engage with on a trial-by-trial basis. Once stable responding was achieved, animals were implanted with jugular vein catheters and allowed evening self-administration of cocaine to determine to what extent these animals engage in drug-taking, as well as how drug-taking influences decision making on morning choice rGT sessions. In the choice rGT, we found that the presence of cues was sufficient to induce decision-making biases, and that a subset of animals were naturally risk preferring. Contrary to our hypotheses, we found that risky rats made less total cocaine infusions than optimal rats. We also found that while risky rats had a greater preference for the cued task at baseline, this preference was diminished after acquisition of cocaine-taking behaviour. This change in task preference was not observed in optimal rats, or in saline self-administering controls. This demonstrates a potential trade-off between two forms of dopamine-evoking tasks: cued gambling and cocaine-taking. Optimal rats may thus consume more cocaine as a result of not receiving adequate dopamine increases through the rGT. In future experiments, the use of this novel behavioural task will allow us to more realistically model the multifaceted impairments seen in human psychiatric disorders.

Disclosures: K. Hrelja: None. **S. Ansary:** None. **E. Chong:** None. **B. Russell:** None. **C.A. Winstanley:** None.

Poster

P679. Neural Bases of Impulsivity and Risky Behavior

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Program #/Poster #: P679.10

Topic: H.03. Decision Making

Support: CIHR Grant F18-03649

Title: Investigating patterns of complex decision-making using a novel behavioural task in male rats

Authors: K. M. HRELJA, ***E. CHONG**, S. ANSARY, B. RUSSELL, C. A. WINSTANLEY; Univ. of British Columbia, Vancouver, BC, Canada

Abstract: The rat gambling task (rGT) closely models human gambling, and accounts for factors like cost-benefit calculations and uncertainty of outcomes. While a majority of animals adopt an optimal decision-making profile in order to maximize reward, a subset of animals trained on the rGT exhibit risky decision-making. Moreover, when trained on a cued variation of the rGT, in which rewards are accompanied with flashy lights and jingles akin to those at casinos, the subset of risk-preferring rats is larger than on the classic rGT. However, experiments to date have never used task variation (i.e. cued or uncued rGT) as a within-subjects variable. It was thus unknown whether or not rats were driven by cued-induced excitement to take more risks, or if they were somehow responding out of stress. We therefore trained the same rats on both versions of these tasks to determine differences on within- and between-subjects variables, as well as task preference. Male Long Evans rats (N=32) were first trained to make nose-poke responses and lever press for (sugar pellet) reward. They were then taught rGT contingencies and to associate one lever with the cued rGT, and the other with the uncued rGT by enforcing forced-choice, before rats could proceed to the free-choice version of the choice rGT. On the free-choice choice rGT rats could freely choose which task and probabilistic outcomes they want to engage with on a trial-by-trial basis. Once stable responding was achieved on this task, animals were implanted with jugular vein catheters and subjected to evening self-administration of cocaine to determine to what extent these animals engage in drug taking, as well as how drug-taking influences their decision making on morning choice rGT sessions. We found the percentage of risk preferring animals on the choice rGT was larger than in experiments where animals were only exposed to the classic rGT, but smaller than cued rGT. Furthermore, the majority of rats preferred to engage with the cued version of the task. We also found that rats took longer to initiate cued trials but were faster to collect reward when compared to uncued trials in the same session. These findings indicate that the presence of salient audiovisual cues is preferred to their absence, and that cues are sufficient to induce decision-making biases on the same rats within the same sessions, underscoring the risk to human gamblers since audiovisual cues often accompany gambling environments.

Disclosures: K.M. Hrelja: None. **E. Chong:** None. **S. Ansary:** None. **B. Russell:** None. **C.A. Winstanley:** None.

Poster

P679. Neural Bases of Impulsivity and Risky Behavior

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Program #/Poster #: P679.11

Topic: H.03. Decision Making

Support: CIHR Project Grant (PJT-162312)

Title: The link between conditioned reinforcers and rodent decision-making on a cued gambling task

Authors: *S. ANSARY, B. RUSSELL, C. A. WINSTANLEY;
Univ. of British Columbia, Vancouver, BC, Canada

Abstract: The salient audiovisual stimuli that are omnipresent in casinos may sustain or exacerbate gambling behaviours in the same way drug-paired stimuli play an important role in maintaining addiction. Cues that predict reward increase the release of dopamine in the brain, and this efflux is maximal when the reward is probabilistic. It has therefore been suggested that both repeated choice of uncertain options, and repeated exposure to cues that predict reward with maximal uncertainty, may predispose individuals to developing an addictive disorder, like gambling disorder, by sensitizing the dopamine system. Previous studies have shown that the addition of reward-concurrent audiovisual cues that increase in complexity with the size of the reward can increase the choice of disadvantageous “high-risk, high-reward” options on the rat gambling task (cued rGT), a rat model of the Iowa Gambling Task (IGT). We therefore hypothesize that 1) increased risky choice on the cued rGT reflects greater sensitivity at baseline to conditioned reinforcement (CRf; i.e., the degree to which a reward-paired cue comes to act as a reinforcer that the rat will work for), and 2) repeated choice of the risky options may itself precipitate a greater ability for reward-paired cued to act as conditioned reinforcers. Subjects were 32 male Long Evans rats. Responding for CRf was tested either before (n = 16) or after (n = 16) acquisition of the cued rGT. In the cued rGT, rats chose between four options associated with distinct magnitudes and probabilities of reward (1-4 sugar pellets) or time-out penalties (5-40s). The optimal strategy was to favour options associated with smaller per trial gains, but shorter penalties. We expect that most rats performing the cued rGT develop a preference for the risky options. We have previously found that higher rates of responding for CRf prior to training on the cued rGT was associated with a better choice strategy, less motor impulsivity, and more trials completed in female rats. We also found that cued rGT training did not increase motivation to respond for CRf. Thus, the results from this study will help elucidate any sex differences in cue-driven behaviour and inform our understanding of how cues influence more complex decision-making in male rats.

Disclosures: S. Ansary: None. **B. Russell:** None. **C.A. Winstanley:** None.

Poster

P679. Neural Bases of Impulsivity and Risky Behavior

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Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P679.12

Topic: H.03. Decision Making

Support: CIHR Grant awarded to Catharine Winstanley

Title: Chronic Cannabis Sativa and Indica Exposure Impairs Decision-Making Strategies in Adult Rodents

Authors: *M. LYSENKO-MARTIN[1], C. P. HUTTON[2], H. BRODIE[1], C. A. WINSTANLEY[3]; [1]Neurosci., Univ. of British Columbia, Vancouver, BC, Canada; [2]Univ. of Victoria, Victoria, BC, Canada; [3]Psychology, Univ. British Columbia, Vancouver, BC, Canada

Abstract: Cannabis is becoming increasingly prevalent in society, with legalization occurring globally at rapid rates. Cannabis impairs decision making and can lead to addiction, particularly when exposure occurs chronically. This impairment can be quantified using a rodent assay of cost-benefit decision making called the cued rat Gambling Task, in which exposure to cue-paired probabilistic rewards triggers neural adaptations that lead to a pro-addiction phenotype. To evaluate the decision-making strategies used by adult rats chronically exposed to *cannabis sativa* and *cannabis indica*, Long Evans male rats were orally dosed daily with 3mg/kg of THC prior to performing the cued rat Gambling Task. Once choice preferences were established, animals underwent a washout period and experimental groups were crossed over and evaluated anew on their decision-making strategies, to account for any impairments THC may have had on learning the task. Animals exposed to *cannabis sativa* were more impulsive and chose riskier strategies early on in daily dosing, after which they slowly learned the optimal choices and aligned with the strategies used by control animals. Animals exposed to *cannabis indica* learned the optimal strategy quicker than *sativa*-exposed animals, but slower than controls. Importantly, both within-subject and between-subject choices were considered. A mixed-effects model was used to evaluate choice preferences, as individual experiences with cost-benefit tasks can vary widely based on whether the prior choice was a win or a loss, and no 2 animals in the same group had an identical decision-making pattern throughout 100+ choices a day for 40+ days of testing. These results suggest that chronic exposure to *cannabis sativa* versus *cannabis indica* can have differential effects on impulsivity, motivation, and decision making behaviors that are indicative of a pro-addiction phenotype.

Disclosures: M. Lysenko-Martin: None. **C.P. Hutton:** None. **H. Brodie:** None. **C.A. Winstanley:** None.

Poster

P679. Neural Bases of Impulsivity and Risky Behavior

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Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P679.13

Topic: H.03. Decision Making

Support: CIHR

Title: Investigating the effects of selective D3 agonist on risk preference in male rats.

Authors: *H. BRODIE, B. RUSSELL, C. WINSTANLEY;
Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Dopamine agonist drugs such as ropinirole are often prescribed to patients with Parkinson's disease to alleviate motor symptoms. However, these drugs often have adverse side effects, and a significant minority of patients go on to develop gambling disorder or other impulse control disorders. Previous research using rodent models established that in healthy male rats, chronic administration of ropinirole can increase risky decision making. This effect is thought to be driven by activation of dopamine receptors. Ropinirole is a D2/D3 agonist and there is evidence of both D2 and D3 receptor involvement in the development of iatrogenic gambling disorders. Accordingly, the purpose of the current experiment was to determine the effects of a selective D3 agonist on risky decision making in male rats using the rodent gambling task (rGT). A cohort of 24 male Long-Evans rats were trained on the cued version of the rGT, a rodent adaptation of the Iowa gambling task commonly used to study risky decision making in humans. Optimal decision making in this task is achieved by preferentially selecting the low risk, low reward options over the high risk, high reward options. Selection of the low risk, low reward options results in fewer timeout penalties and more sugar pellets awarded over time. Audiovisual cues were paired with rewards and have been shown to increase risk preference. Rats were dosed with saline(n=12) or 0.1 mg/kg of D3 selective agonist PD128907(n=12) ten minutes before completing the task. Rat were dosed throughout acquisition of the task and until they reached a stable behavioural baseline (32 sessions). Rats then completed an additional 8 sessions in washout. Results showed that although the D3 agonist significantly increased the number of omitted trials and latency to choose an option while decreasing the number premature responses, risk preference was not significantly affected. The effects on omissions, choice latency and premature responses disappeared immediately in washout. These findings offer new insights into the effects of ropinirole on risky decision making and suggest that D3 receptor activation alone may not be responsible for the development of iatrogenic gambling disorder.

Disclosures: H. Brodie: None. **B. Russell:** None. **C. Winstanley:** None.

Poster

P723. Addictive Drugs: Developmental Effects III

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Program #/Poster #: P723.01

Topic: G.09. Drugs of Abuse and Addiction

Support: NIGMS Grant SC2GM109811
NIGMS Grant SC3GM130467

Title: Autophagy induction and accumulation of phosphorylated tau in the hippocampus and prefrontal cortex of adult male C57BL/6 mice exposed to adolescent fluoxetine treatment

Authors: *M. RODRIGUEZ, O. LIRA, F. J. FLORES RAMIREZ, S. D. IÑIGUEZ; Psychology, The Univ. of Texas at El Paso, El Paso, TX

Abstract: Fluoxetine (FLX) represents the antidepressant of choice for the management of pediatric mood-related illnesses. Accumulating preclinical evidence suggests that ontogenic FLX exposure leads to deregulated affect-related phenotypes in adulthood. Mood-related symptomatology constitutes a risk factor for various neurological disorders, including Alzheimer's disease (AD), making it possible for juvenile FLX history to exacerbate the development of neurodegenerative diseases. Because AD is characterized by the pathological accumulation of hyperphosphorylated Tau - which can result from impaired function of protein degradation pathways, such as autophagy and the ubiquitin-proteasome system (UPS), we evaluated the long-term effects of adolescent FLX exposure on these pathways, using mice as a model system. To do this, we subjected C57BL/6 adolescent male mice to FLX (20 mg/kg) during the adolescence stage of development (postnatal days [PD] 35-49). After a 21-day washout period, mice were euthanized (PD70) and, using immunoblotting analysis, we evaluated protein markers of autophagy (Beclin-1, LC3-II, p62) and the UPS (K48-pUb), as well as AD-associated forms of phosphorylated Tau, within the hippocampus and prefrontal cortex. We found that juvenile FLX mediated a long-term increase in the expression of autophagy protein markers (LC3-II and p62) in a manner that is consistent with autophagy activation, particularly in the prefrontal cortex. Furthermore, FLX history induced persistent accumulation of AD-associated variants of Tau in both the hippocampus and prefrontal cortex. Overall, our data suggest that adolescent FLX treatment may have enduring effects in the neuronal protein degradation machinery, which could adversely influence the clearance of abnormal proteins, potentially predisposing individuals to develop AD in later life.

Disclosures: M. Rodriguez: None. **O. Lira:** None. **F.J. Flores Ramirez:** None. **S.D. Iñiguez:** None.

Poster

P723. Addictive Drugs: Developmental Effects III

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Program #/Poster #: P723.02

Topic: G.09. Drugs of Abuse and Addiction

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SC1-GM127251

Title: Female rats display greater nicotine withdrawal-induced cellular activation of a central portion of the interpeduncular nucleus versus males

Authors: *V. E. ESPINOZA[1], F. MATOS[1], P. CORREA[1], A. M. KHAN[2], L. E. O'DELL[1]; [1]Dept. of Psychology, [2]Dept. of Biol. Sci. and Border Biomed. Res. Ctr., The Univ. of Texas at El Paso, El Paso, TX

Abstract: *Background:* The *interpeduncular nucleus (>1840)* (IPN) has been shown to modulate the behavioral effects of nicotine withdrawal in male rodents. To date, the contribution of this brain structure to sex differences in withdrawal is largely unexplored. *Methods:* This study compared neuronal activation, as reported by observable Fos expression in the IPN of nicotine-dependent female and male rats experiencing withdrawal. We provisionally localized the Fos-expressing cells to certain IPN subnuclei within Swanson's standardized brain atlas (2018). Adult female and male rats were prepared with a pump that delivered nicotine (3.2 mg/kg/day; base) continuously. Controls received a sham surgery. Fourteen days later, the rats received administration of saline or the nicotinic receptor antagonist, mecamylamine (3.0 mg/kg; salt), and physical signs and anxiety-like behavior were assessed. The rats were then euthanized and brain sections containing the IPN were processed for Fos immunofluorescence to infer the possible IPN subnuclei displaying differential activation between sexes. *Results:* Both female and male rats displayed withdrawal-induced Fos expression within the IPN. Compared to males, female rats displayed greater numbers of withdrawal-induced Fos-positive cells within a circumscribed portion of the IPN that may fall within the cytoarchitectural boundaries of the *central subnucleus (>1840)* (IPNc). The withdrawal-induced activation of the IPN was correlated with negative affective states in females, but not males. *Conclusion:* These data suggest that that a centrally located group of IPN cells, presumably situated partly or completely within the IPNc, play a role in modulating sex differences in negative affective states produced by withdrawal.

Disclosures: V.E. Espinoza: None. **F. Matos:** None. **P. Correa:** None. **A.M. Khan:** None. **L.E. O'Dell:** None.

Poster

P723. Addictive Drugs: Developmental Effects III

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Program #/Poster #: P723.03

Topic: G.09. Drugs of Abuse and Addiction

Title: Alprazolam exposure during adolescence induces life-long dysregulation of reward sensitivity and second messenger signaling within the nucleus accumbens (NAc).

Authors: *A. CARDONA-ACOSTA[1], O. K. SIAL[2], T. GNECCO[1], G. ENRIQUEZ MARTI[1], D. HERNANDEZ-GUZMAN[1], C. A. BOLANOS-GUZMAN[2];
[2]Psychological and Brain Sci., [1]Texas A&M Univ., College Station, TX

Abstract: Benzodiazepines are prescribed widely as anxiolytics, hypnotics, muscle relaxants, and anticonvulsants. However, their utility is limited by unwanted side effects such as abuse liability and the potential for dependence. Benzodiazepine-related emergency room visits have increased in the US within the past two decades, and despite concerns surrounding their use, there has been a substantial increase in their prescription rates. Benzodiazepines are commonly abused concurrently with opioids, resulting in greater psychopathology and complex comorbidities. There is evidence of increased use and abuse of alprazolam (Xanax; ALP) during adolescence, yet most available neurobiological evidence has been derived from studies using adult organisms. This study was designed to investigate the behavioral and molecular consequences of ALP exposure during adolescence. Adolescent C57BL/6J male mice (postnatal day [PD] 35) were pretreated with ALP (0, 0.5, 1.0 mg/kg) once daily from PD35-49. Changes in behavioral responsiveness to morphine (2.5 and 5.0 mg/kg), using the conditioned place preference paradigm (CPP) were assessed 24 h (short-term) and one-month (long-term) after the end of ALP treatment. In a separate experiment, male mice were treated with ALP (0.0, 0.5 mg/kg) from PD35-49 and then sacrificed 24 h (short term) or 1-month (long-term) after the last injection to assess levels of extracellular signal regulated kinase1/2 (ERK1/2) protein phosphorylation within the nucleus accumbens (NAc). Our results show that ALP-pretreated mice developed a strong preference to the CPP compartment paired with a subthreshold dose (2.5 mg/kg) of morphine (short-term), and this effect was also present in the long-term group (a month after ALP exposure). In addition, we report an increase (short-term) followed by a decrease in ERK1/2 protein phosphorylation (long-term) within the NAc. Ongoing studies are assessing protein phosphorylation of ERK's downstream targets such as the cAMP Response Element-Binding Protein (CREB) and Protein Kinase B (AKT). Overall, these findings indicate that exposure to ALP during periods prior to adulthood potentiates the rewarding effects of drugs of abuse such as morphine, and that ALP exposure during this period results in persistent changes in ERK-signaling within the NAc, a brain region implicated in regulation of both drug reward and mood-related disorders.

Disclosures: A. Cardona-Acosta: None. **O.K. Sial:** None. **T. Gnecco:** None. **G. Enriquez Marti:** None. **D. Hernandez-Guzman:** None. **C.A. Bolanos-Guzman:** None.

Poster

P723. Addictive Drugs: Developmental Effects III

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIGMS Grant SC2GM109811
NIGMS Grant SC3GM130467

Title: Adolescent fluoxetine exposure induces persistent gene expression changes in the hippocampus of adult male C57BL/6 mice

Authors: *A. THEMANN, O. LIRA, F. J. FLORES RAMIREZ, S. D. IÑIGUEZ;
Psychology, The Univ. of Texas at El Paso, El Paso, TX

Abstract: Fluoxetine (FLX), a selective serotonin reuptake inhibitor, is the first line of pharmacological intervention in pediatric patients suffering from affect-related illnesses, such as anxiety and depression. Although the use of this antidepressant treatment has been deemed efficacious in the adolescent population, the enduring neurobiological consequences of juvenile FLX exposure are not well understood. Thus, we explored for persistent molecular adaptations in the adult hippocampus, as a function of adolescent FLX pretreatment. To do this, we administered daily peritoneal injections of FLX (20 mg/kg/day) to male C57BL/6 mice during adolescence (postnatal day [PD] 35-49). After a 21-day washout period (PD70), whole hippocampal tissue was dissected, and subsequent qPCR analysis was ran to assess changes in the expression of genes associated with neuronal survival. Specifically, we evaluated major intracellular signal transduction pathways, including the Ras-mitogen-activated protein kinase (MAPK), phosphatidylinositide-3-kinase (PI3K)/AKT pathway, as well as several transcription factors. Our results indicate that adolescent FLX treatment results in a long-term upregulation of mRNA levels across numerous genes from the ERK, and PI3K/AKT pathways, along with increases of the transcription factors CREB, ΔFosB, and zif268. Additionally, adolescent FLX treatment resulted in persistent increases of transcripts associated with cytoskeletal integrity (β-actin) and caspase activation (DIABLO), while decreasing genes associated with metabolism (fucose kinase) and overall neuronal activation (c-Fos). Collectively, these data indicate that adolescent FLX exposure mediates persistent alterations in hippocampal gene expression in adulthood, thus, questioning the safety of early-life exposure to this antidepressant medication.

Disclosures: A. Themann: None. **O. Lira:** None. **F.J. Flores Ramirez:** None. **S.D. Iñiguez:** None.

Poster

P723. Addictive Drugs: Developmental Effects III

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Title: Adolescent and adult rats exhibit context-dependent methamphetamine sensitization while preweanling rats fail to exhibit sensitization using a one-trial procedure.

Authors: *F. S. OMERJEE, G. C. VERDUZCO, B. SORTMAN, C. MORENO-BARRIGA, E. NUÑEZ-LARIOS, D. FRANCO, A. R. ZAVALA;
California State University, Long Beach, Long Beach, CA

Abstract: Behavioral sensitization is a phenomenon in which previous administration of a psychostimulant drug elicits a heightened behavioral response in subsequent exposures. Even one previous pairing is enough to elicit sensitization, known as a one-trial or two-shot design. In this paradigm, adult rats exhibit a sensitized response only evident in the area in which they previously received the psychostimulant. This is known as context-dependent sensitization. Preweanling rats (postnatal day [PD] 24 or younger), however, exhibit context-independent sensitization, which is a heightened response regardless of what environment the drug was associated. Interestingly, adolescent rats (PD 25-50) do not exhibit a sensitized response when exposed to methamphetamine (METH). Prior studies examining the preweanling and adolescent stage have used a wide range of doses, but doses lower than 1 mg/kg METH have not been used. Thus, the present study examined one-trial sensitization in preweanling, adolescent, and adult rats using 0.1 and 0.3 mg/kg of METH as well as an additional 1.0 mg/kg dose of METH for adult rats. The study also sought to determine the role of context in moderating a sensitized response across development. During the two-day study, PD 18, 28, 38, or 68 male and female rats were pretreated with saline or 3 mg/kg METH and placed in a novel activity chamber where their locomotor activity (distance traveled in m) was assessed for 60 min. Rats were then taken to their home cage and, based on the assigned groups, injected with saline or 3 mg/kg METH 45 min later and placed back in their home cage. During the challenge test, PD 19, 29, 39, or 69 male and female rats were injected with saline or either 0.1 or 0.3 mg/kg METH (also 1.0 mg/kg for PD 69) before being placed in the activity chamber, during which distance traveled was assessed for 90 min. The study results found that adolescent rats (PD 29 and PD 39) and adult rats (PD 69) exhibited context-dependent sensitization. However, preweanling rats did not exhibit behavioral sensitization at any dose. Interestingly, sex-related differences were observed as PD 29 and PD 39 female rats exhibited behavioral sensitization with both challenge doses of METH. In contrast, male rats exhibited behavioral sensitization only at 0.1 mg/kg METH. Contrary to prior studies, both groups of adolescent rats exhibited context-dependent

sensitization using low doses of METH. These findings highlight the need to examine a wide range of doses and different age periods to understand the development of sensitization in rats.

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Poster

P723. Addictive Drugs: Developmental Effects III

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Program #/Poster #: P723.06

Topic: G.09. Drugs of Abuse and Addiction

Support: NIGMS Grant SC3GM130467
NIGMS Grant SC2GM109811

Title: Enduring effects of juvenile ketamine exposure for the rewarding properties of cocaine and sucrose in mice

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Abstract: Ketamine, a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, has shown rapid antidepressant efficacy in adolescent treatment-resistant depressed patients. However, the potential enduring side effects of ketamine exposure during early development have not been explored. Thus, using male and female C57BL/6 mice, we examined whether exposure to ketamine during adolescence, alone (Experiment 1) or in combination with stress (Experiment 2), results in long-lasting changes in sensitivity to the rewarding properties of cocaine and sucrose in adulthood. Specifically, in Experiment 1, mice received either ketamine (20 mg/kg) or saline (VEH) for 15 consecutive days during adolescence (Postnatal Day [PD] 35-49). Twenty-one days after ketamine exposure, once mice reached adulthood (PD70), we assessed their behavioral responsivity to a two-bottle choice sucrose (1%) test or cocaine (0, 5, 10 mg/kg) place preference (CPP) experiment. Our results showed that adult mice pre-treated with ketamine during adolescence displayed an enhanced preference for sucrose and environments paired with cocaine in male, but not female, mice. Thus, in Experiment 2, using male mice only, we evaluated whether adolescent ketamine exposure (PD35-44), in combination with social defeat or the vicarious defeat stress paradigms, would influence responses to sucrose or cocaine preference later in life (PD70). Here, no long-term differences in sucrose or cocaine preference were evident as a function of juvenile stress-and-ketamine pretreatment, in male mice. Together, our findings suggest that in the absence of social stressors, juvenile exposure to

ketamine alters sensitivity to the rewarding properties of non-drug (sucrose) and drug (cocaine) reward-related stimuli, in a sex-dependent manner, later in life.

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Poster

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Topic: G.09. Drugs of Abuse and Addiction

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Title: Sex- and dose-dependent differences in preference for ethanol in preadolescent and adolescent rats

Authors: *T. M. NELSON[1], T. A. GONZALEZ[1], A. K. GARCIA[1], D. Q. PHAM[1], D. FRANCO[1], E. A. BATES[1], U. M. RICOY[2], S. D. IÑIGUEZ[3], A. R. ZAVALA[1];
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Abstract: Ethanol is the drug of choice among adolescents in the United States. Adolescence is a pivotal period in brain development making young people specifically at risk for a variety of different consequences as a result of ethanol use. Previous literature using the conditioned place preference (CPP) paradigm, a validated animal model of drug reward, has revealed the difficulty of reliably demonstrating ethanol preference in adolescent rats, potentially due to sex and dose-related differences. Therefore, we hypothesized that male and female adolescent rats would exhibit dose-dependent differences in ethanol preference. Rats underwent a 10-day CPP procedure to assess ethanol preference during preadolescence (experiment 1) and adolescence (experiments 2 and 3). On day 1, initial preference for a two-chamber CPP apparatus was assessed during 15 min (experiments 1 and 2) or 20 min (experiment 3) sessions. On days 2-9, rats were conditioned with saline in their initially preferred chamber or ethanol in their initially non-preferred chamber on alternating days for 15 min. On day 10, the preference for the ethanol-paired chamber was assessed using identical procedures to the first day of the experiment. In experiments 1 and 2, rats were randomly assigned to receive an injection of ethanol (0.0, 0.5, 1.0, or 2.0 g/kg, IP) before being placed in the ethanol-paired chamber. In experiment 3, rats were

randomly assigned to receive an injection of ethanol (0.0, 0.0156, 0.0313, 0.0625, 0.125, 0.5, or 2.0 g/kg, IP) before being placed in the ethanol-paired chamber. In experiment 1, both male and female rats demonstrated ethanol-induced CPP when administered the highest dose of ethanol (2.0 g/kg). In experiment 2, sex differences emerged as female rats continued to prefer a high dose of ethanol (2.0 g/kg) and male rats demonstrated a preference for the ethanol-paired chamber with the lowest ethanol dose (0.5 g/kg). In experiment 3, male rats demonstrated a robust preference for ethanol when administered low ethanol doses (0.0625, 0.125 g/kg). Overall, sex differences did not emerge in preadolescence because rats exhibited a similar preference for ethanol, regardless of sex. However, during adolescence, a shift in preference was seen in male rats that demonstrated ethanol-induced CPP at low doses of ethanol. In conclusion, ethanol-induced CPP can be reliably demonstrated in adolescent rats, which can aid in a better understanding of the underlying brain mechanisms involved in adolescent ethanol use.

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Poster

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Program #/Poster #: P723.08

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant GM136467

Title: Reciprocal cross-sensitization between cocaine and RU 24969

Authors: *J. A. M. ROBINSON, D. C. GLEASON, L. L. COTTER, S. A. MCDOUGALL; Dept. of Psychology, California State Univ., San Bernardino, CA

Abstract: Cross-sensitization occurs when a drug causes an enhanced behavioral response after a pretreatment regimen with a different drug. The ability of drugs to cross-sensitize is often used as evidence that a common neural substrate underlies the sensitization process. Cocaine, amphetamine, and methamphetamine are the psychostimulant compounds most typically used to induce behavioral sensitization, but indirect and direct serotonin (5-HT) agonists can also produce locomotor sensitization. The goal of the present study was to use the cross-sensitization paradigm to determine whether repeated treatment with the nonselective 5-$HT_{1A/1B}$ receptor agonist RU 24969 produces the neuronal changes necessary for behavioral sensitization. The preweanling rat model was used, because it has proven useful for elucidating drug action in a variety of behavioral and physiological assays. In a series of experiments, preweanling rats were pretreated with RU 24949 for four consecutive days and then tested with a psychostimulant drug

(cocaine or methamphetamine). Reciprocal cross-sensitization was also assessed (i.e., rats were pretreated for four days with a psychostimulant drug and challenged with RU 24969). In addition to the multi-trial procedure, reciprocal cross-sensitization was examined using a one-trial sensitization procedure (i.e., rats were pretreated with a psychostimulant on a single day and then challenged with RU 24969 two days later). Using the multi-trial procedure, reciprocal cross-sensitization between cocaine and RU 24969 was evident, whereas methamphetamine and RU 24969 did not cross-sensitize. When a one-trial pretreatment regimen was used, cross-sensitization was only detected when preweanling rats were pretreated with RU 24969 and tested with cocaine, but not the reverse. In sum, the present results show that the nonselective 5-$HT_{1A/1B}$ receptor agonist RU 24969 cross-sensitizes with cocaine, but not methamphetamine, in the preweanling rat. This dichotomy may be a function of cocaine having a greater affinity for the serotonin transporter than methamphetamine. Nonetheless, the present evidence suggests that the behavioral sensitization produced by repeated cocaine and RU 24969 administration shares a common neural substrate.

Disclosures: J.A.M. Robinson: None. **D.C. Gleason:** None. **L.L. Cotter:** None. **S.A. McDougall:** None.

Poster

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Program #/Poster #: P723.09

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant GM136467

Title: Importance of the serotonin 5-HT_{1B} receptor for the locomotor activity of preweanling and adult male and female rats

Authors: *L. L. COTTER, M. J. ROE, J. A. M. ROBINSON, D. C. GLEASON, D. J. GONZALEZ, S. A. MCDOUGALL;
Dept. of Psychology, California State Univ., San Bernardino, CA

Abstract: 5-$HT_{1A/1B}$ receptor agonists (e.g., RU 24969), as well as 5-HT_{1A} receptor agonists (e.g., 8-OH-DPAT), increase the locomotor activity of both preweanling and adult rodents. Sex differences, are apparent, as adult female mice are more sensitive to the locomotor activating properties of RU 24969 than adult male mice. The role played by the 5-HT_{1B} receptor in locomotion is less certain, and preliminary evidence suggests that the actions of 5-HT_{1B} receptor agonists are not uniform across ontogeny. To more fully examine the ontogeny of the 5-HT_{1B} receptor system, the locomotor activity and axillary temperatures of preweanling and adult male and female rats were assessed in two experiments. In the first experiment, preweanling (PD 10

and PD 15) and adult (PD 70) male and female rats were injected with a broad dose range of the 5-HT_{1B} agonist CP 94253 (2.5-10 mg/kg) immediately before locomotor activity testing (axillary body temperatures were also recorded). In the second experiment, specificity of drug action was determined in preweanling rats (PD 10) by administering saline, WAY 100635 (a selective 5-HT_{1A} antagonist), or GR 127935 (a 5-HT_{1B} antagonist) 15 min before CP 94253 (10 mg/kg) treatment. Results showed that all doses of CP 94253 significantly increased the locomotor activity of preweanling rats on PD 10, but CP 94253 did not increase the locomotor activity of older preweanling rats (PD 15). CP 94253 significantly decreased the locomotor activity of both male and female adult rats. The ability of CP 94253 to increase the locomotion of PD 10 rats was due to actions at the 5-HT_{1B} receptor, because GR 127935 (a 5-HT_{1B} antagonist), but not WAY 100635, fully attenuated the CP 94253-induced locomotor activity. Regardless of age, CP 94253 (2.5-10 mg/kg) significantly reduced the axillary temperatures of preweanling and adult male and female rats. When considered together with past results, this study shows that the 5-HT_{1B} receptor system mediates the locomotor activity of male and female preweanling rats during early ontogeny; however, the 5-HT_{1B} receptor plays a significantly reduced role with increasing age. The serotonin neurotransmitter system in general, as well as the 5-HT_{1B} receptor system, shows substantial maturational changes across early ontogeny, adolescence, and into adulthood. It is likely that one or more of these ontogenetic changes is responsible for the age-dependent differences in locomotor responsiveness to CP 94253.

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Poster

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Program #/Poster #: P723.10

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant GM136467

Title: Repeated treatment with 5-HT_{1A} and 5-HT_{1B} receptor agonists: evidence of tolerance and behavioral sensitization

Authors: *M. J. ROE, J. A. TAYLOR, C. J. GREENWOOD, S. A. MCDOUGALL;
Dept. of Psychology, California State Univ., San Bernardino, CA

Abstract: Repeated treatment with serotonin (5-HT) and dopamine (DA) agonists can cause a progressive augmentation of locomotor activity called behavioral sensitization. On the other hand, repeated treatment with the selective 5-$HT_{1A/1B}$ receptor agonist RU 24969 causes a pronounced tolerance response. Specifically, acute administration of RU 24969 produces

locomotion, motoric disturbances, and hypothermia in preweanling and adult rats: effects that weaken or are eliminated with repeated daily treatment. Because tolerance could potentially mask expression of behavioral sensitization, it is uncertain whether repeated stimulation of 5-$HT_{1A/1B}$ receptors produces the neurobiological changes necessary for sensitized responding. To further examine the role of 5-HT_{1A} and 5-HT_{1B} receptors in the development of tolerance and behavioral sensitization, preweanling rats were repeatedly treated with the selective 5-HT_{1A} receptor agonist 8-OH-DPAT and/or the selective 5-HT_{1B} receptor agonist CP 94253. Locomotor activity and axillary temperatures were measured. In a series of three experiments, male and female preweanling rats were injected with saline or the selective 5-HT_{1A} agonist 8-OH-DPAT (0.1-8 mg/kg) on PD 20. On PD 22, rats previously administered saline were injected with 8-OH-DPAT (0.1-8 mg/kg), whereas rats initially given 8-OH-DPAT were injected with the same dose of 8-OH-DPAT again. Identical experiments were conducted using the selective 5-HT_{1B} agonist CP 94253 (2.5-20 mg/kg) or combined treatment with 8-OH-DPAT (4 mg/kg) + CP 94253 (5 mg/kg). Immediately after drug injections, rats were placed in activity chambers and distance traveled was measured for 45 min (axillary temperatures were also recorded). On the pretreatment day, 8-OH-DPAT, CP 94253, and DPAT+CP increased locomotor activity and caused hypothermia. Repeated treatment with 8-OH-DPAT (2 or 8 mg/kg) or DPAT+CP caused locomotor sensitization in preweanling rats, because rats pretreated and tested with these compounds exhibited more locomotor activity on the test day than rats given the same drugs on only the test day. In contrast, tolerance to the hypothermic effects of 8-OH-DPAT (8 mg/kg), CP 94253 (5-20 mg/kg), and DPAT+CP was evident after repeated drug treatment. These results show that repeated treatment with 8-OH-DPAT causes both tolerance (hypothermia) and sensitization-like effects (augmented locomotor activity) in preweanling rats. Although CP 94253 increased locomotor activity and produced hypothermia in preweanling rats, repeated treatment with CP 94253 only produced a tolerance response (axillary temperatures) and not behavioral sensitization.

Disclosures: M.J. Roe: None. **J.A. Taylor:** None. **C.J. Greenwood:** None. **S.A. McDougall:** None.

Poster

P738. Alcohol: Circuitry and Neurophysiology of Addiction I

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Program #/Poster #: P738.01

Topic: G.09. Drugs of Abuse and Addiction

Support: Department of Veterans Affairs Merit Research Award I01BX002661
NIH Grant AA028175-01

Title: Activation of dopamine D2 receptors in the medial shell region of the nucleus accumbens increases Per1 expression to enhance alcohol consumption

Authors: *V. MISHRA, M. PARIKH, A. SONI, R. SHARMA, P. SAHOTA, M. THAKKAR; Neurol., Univ. of Missouri, Columbia, MO

Abstract: Circadian genes, including Per1, in the medial shell region of nucleus accumbens (mNAcSh), regulate binge alcohol consumption. However, the upstream mechanism regulating circadian genes-induced alcohol consumption is not known. Since activation of dopamine D2 receptors (D2R) increases Per1 gene expression, we hypothesized that local infusion of quinpirole, a D2R agonist, by increasing Per1 gene expression in the mNAcSh, will increase binge alcohol consumption in mice. We performed two experiments on male C57BL/6J mice, instrumented with bilateral guide cannulas above the mNAcSh, and exposed to a four-day drinking-in-dark (DID) paradigm. The first experiment determined the effects of bilateral infusion of quinpirole (100 ng/300 nl/site) or DMSO (Vehicle group) in the mNAcSh on Per1 gene expression and alcohol consumption. The second experiment determined the effect of antisense-induced downregulation of Per1 in the mNAcSh on the quinpirole-induced increase in alcohol consumption. Control experiments were performed by exposing the animals to sucrose (10% w/v). After the experiment, animals were euthanized, brains removed, and processed for localization of injection sites and analysis of Per1 gene expression in the mNAcSh. As compared to the DMSO, local bilateral infusion of quinpirole significantly increased the expression of Per1 in the mNAcSh along with an increase in the amount of alcohol consumed in mice exposed to DID paradigm. In addition, local antisense-induced downregulation of Per1 significantly attenuated the effects of intro-accumbal infusion of quinpirole on alcohol consumption. Our results suggest that Per1 in the mNAcSh mediates D2R activation-induced increase in alcohol consumption.

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Poster

P738. Alcohol: Circuitry and Neurophysiology of Addiction I

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Topic: G.09. Drugs of Abuse and Addiction

Support: Department of Veterans Affairs Merit Research Award I01BX002661
NIH Grant AA028175-01

Title: Antisense-induced downregulation of circadian genes in the medial shell region of the nucleus accumbens reduces alcohol consumption, in mice, exposed to chronic excessive alcohol consumption

Authors: *R. SHARMA, V. MISHRA, M. PARIKH, A. SONI, P. SAHOTA, M. THAKKAR; Research, Neurol., Truman VA Med. Center/University of Missouri, Columbia, MO

Abstract: Background: Circadian genes, present in the nucleus accumbens, are implicated in a variety of psychiatric disorders including anxiety, stress, reward and substance abuse, however, their role in chronic alcohol abuse has not been investigated. We hypothesized that antisense-induced knockdown of major circadian genes (Per1, Per2 and NPAS2) in the mNAcSh will reduce alcohol consumption and preference in mice exposed to intermittent access 2-bottle choice (IA2BC) paradigm, a model for alcohol use disorders. **Methods:** Adult male C57BL/6J mice (N = 14), surgically implanted with bilateral guide cannulas above the mNAcSh, were exposed to alcohol (20% v/v) or saccharin (0.03%). In the fourth week, after alcohol consumption was stabilized, a mixture of antisense (AS-ODNs; Antisense; N = 7) or nonsense oligodeoxynucleotides (NS-ODNs; Nonsense; N = 7) against circadian genes, were bilaterally infused into the mNAcSh. Subsequently, alcohol/saccharin consumption and preference were monitored for 18 h. On completion, animals were euthanized and brains removed. Localization of injection sites and verification of circadian gene downregulation was performed by selecting sections containing microinjection sites. Tissue punches were obtained and processed for RT-PCR to verify antisense-induced gene knockdown in the mNAcSh. **Results**: Histological and/or visual verification of injection sites suggested that all infusion sites were localized in the mNAcSh. As compared to NS-ODNs, AS-ODNs infusion significantly reduced the expression of all three circadian genes along with the reduction in alcohol consumption and preference without affecting saccharin consumption and preference. **Conclusions:** Our results suggest that circadian genes in the mNAcSh may have a causal role in alcohol consumption.

Disclosures: R. Sharma: None. **V. Mishra:** None. **M. Parikh:** None. **A. Soni:** None. **P. Sahota:** None. **M. Thakkar:** None.

Poster

P738. Alcohol: Circuitry and Neurophysiology of Addiction I

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA R01AA027768 (J.W.)
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Title: D1-msn ensembles in the dorsomedial striatum positively regulate alcohol-seeking and relapse

Authors: T. TAN[1], J. LU[1], X. XIE[1], W. WANG[1], W. PURVINES[2], K. WOODSON[1], J. BONIFACIO[1], K. BEGO[1], X. WANG[1], J. WANG[1];
[1]Dept. of Neurosci. and Exptl. Therapeut., Texas A&M Hlth. Sci. Ctr., Bryan, TX; [2]NEXT, Texas A&M Univ., Bryan, TX

Abstract: The dorsomedial striatum (DMS) is essential for the reinforcement of addictive behaviors. The involvement of dopamine D1 receptor-expressing medium spiny neurons (D1-MSNs) in the DMS is increasingly being appreciated for mediating reinforcement behavior in addiction, and enhanced excitatory synaptic transmission has been found in D1-MSNs after chronic alcohol drinking. In this study, we found excessive ethanol intake strengthened cortico-striatal glutamatergic transmission selectively in DMS D1-MSNs. Using triple transgenic FosTRAP; D1-tdTomato; Snap25-EGFP mice in which D1-MSNs expressed tdTomato and activated neurons expressed EGFP after tamoxifen-induced Cre recombination, we discovered more striatal D1-MSNs were activated in ethanol-drinking mice than their water control under the two-bottle choice (2BC) procedure. Additionally, we observed a greater cortico-striatal transmission in alcohol-activated D1-MSN ensembles than non-activated D1-MSNs. Notably, another experiment using ArcTRAP animals found more alcohol-activated neuronal ensembles in the DMS than in other striatal regions. Consistent with these physiological and histological results, behavioral studies found that chemogentic inhibition of trapped D1MSN-2BC ensembles suppressed the alcohol consumption during the 2BC test even in the presence of quinine, suggesting alcohol-activated neurons also contribute to the compulsive alcohol consumption. Similar results were found in ArcTRAP mice trained with operant alcohol self-administration (SA): chemogenetic inhibition of the trapped D1-MSN-SA ensembles decreased both alcohol-seeking and relapse. Interestingly, chemogenetic inhibition D1-MSN-SA ensembles also decreased alcohol consumption under the 2BC test in the presence or absence of quinine. Conversely, chemogenetic inhibition of D1-MSN-2BC ensembles suppressed the subsequent SA seeking and relapse. These findings indicate that *ad libitum* alcohol experience during the 2BC may share at least part of neuronal ensembles with the SA addictive behaviors. In summary, our results demonstrate that DMS D1-MSNs are activated by alcohol consumption (both *ad libitum* and SA) and contribute to compulsive drinking, alcohol-seeking, and relapse. This study also suggests that targeting inhibition of the D1-MSN ensembles may help to alleviate alcohol use disorders.

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Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant AA028175-01
Department of Veterans Affairs Merit Research Award I01BX002661

Title: Genetic ablation of cholinergic interneurons in the medial shell region of the nucleus accumbens reduces binge drinking in C57BL/6J mice

Authors: *A. SONI, R. SHARMA, V. MISHRA, M. PARIKH, P. SAHOTA, *M. THAKKAR; Truman VA Med. Center/University of Missouri, Columbia, MO, Columbia, MO

Abstract: Background: Alcohol use disorder (AUD) is a chronic relapsing brain disorder responsible for serious health and economic consequences in the U.S. Apart from genetic predisposition, binge drinking or heavy alcohol consumption is a leading cause of AUD, hence it is important that we understand the mechanism of alcohol consumption to treat AUD. Cholinergic interneurons (CIN) present in the nucleus accumbens NAc have been strongly implicated in substance abuse including cocaine, however, their role in alcohol abuse has not been investigated. We hypothesized that genetic ablation of CIN in the medial shell region of the NAc (mNAcSh) will reduce alcohol consumption in mice exposed to alcohol via the drinking-in-dark (DID) paradigm. **Methods:** To test our hypothesis. ChAT-cre mice (C57BL/6J transgenic mice expressing Cre-recombinase in the cholinergic neurons) were used in this study. Mice were bilaterally infused either with taCasp3-encoding AAV (serotype 8) containing EF1a for cre-dependent, bicicstronic expression of designer pro-taCasp3 and TEVp (**AAV-flex-taCasp3-TEVp; taCasp3 group)** or control virus (Ns, **AAV-EF1a-DIO**; 500nL/side; **Control group**) in mNAcSh and left them undisturbed for two weeks to allow for the expression of taCasp3 in the CIN. Next, mice were subjected to binge alcohol consumption using the 4-day DID paradigm. Alcohol consumption was measured for 2 h (Days1-3) and 4 h (Day 4). Blood alcohol concentration was measured after alcohol consumption on Day 4. Separate groups of mice were used to examine the effects of CIN ablation on sucrose (10% w/v; control for calorie and taste) consumption using the same paradigm. Microinfusion sites and the loss of cholinergic neurons were verified using choline acetyltransferase (ChAT) immunohistochemistry (IHC). **Results:** Infusion of taCasp3 caused a significant reduction (~50%) in the number of CIN in the mNAcSh as revealed by ChAT IHC. In addition, as compared to the Controls, mice in the taCasp3 group displayed a significant reduction in the amount of alcohol consumed during four hours of binge drinking on day 4 of the DID paradigm. No differences were found in sucrose consumption. **Conclusions:** Our results strongly suggest that CIN neurons in the mNAcSh are responsible for binge drinking in mice.

Disclosures: A. Soni: None. **R. Sharma:** None. **V. Mishra:** None. **M. Parikh:** None. **P. Sahota:** None. **M. Thakkar:** None.

Poster

P738. Alcohol: Circuitry and Neurophysiology of Addiction I

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Support: Department of Veterans Affairs Merit Research Award I01BX002661
NIH Grant AA028175-01

Title: Adenosine A2a receptor in accumbal shell region of nucleus accumbens modulate per 1 expression to alter the alcohol consumption in mice

Authors: *M. PARIKH, R. SHARMA, V. MISHRA, A. SONI, M. THAKKAR, P. SAHOTA; Truman V.A. Med. Center/University of Missouri, Columbia, MO

Abstract: INTRODUCTION: Adenosine via its receptors (A1R and/or A2R) mediates most of the behavioral effects of alcohol such as sleep promotion via A1R in the basal forebrain, motor incoordination via A2aR in the cerebellum etc. Evidence from studies using systemic pharmacological interventions or global knockout animals suggests that adenosine via adenosine A2 receptors (A2a) attenuates the rewarding and reinforcing effects of alcohol. However, the underlying neuroanatomical mechanism is not clear. Circadian genes including Per1 gene in the medial shell region of nucleus accumbens (mNAcSh) play a key role in alcohol consumption, hence we hypothesized that local A2a activation in the mNAcSh will reduce Per1 gene expression in the mNAcSh resulting in a reduction in the alcohol consumption.**METHODS:** To test our hypothesis, we surgically implanted bilateral guide cannulas above the mNAcSh in male C57BL/6J mice. After surgical recovery, mice were exposed to four-days Drinking-in-the-dark (DID) paradigm. On day, one hour prior to alcohol consumption, mice were infused with either A2a agonist, CGS 21680 (50 ng/site) or DMSO (vehicle), bilaterally in the mNAcSh. Subsequently, the amount of alcohol consumed during 4 h of alcohol access was examined. At the end of alcohol consumption, animals were euthanized, brains removed and processed for examination of Per1 gene expression by RT-PCR. Control experiments were performed by exposing the animals to sucrose [10% (w/v taste and calorie].
RESULTS: Compared to controls, mice administered with CGS 21680 displayed a significant reduction in the alcohol consumption without affecting sucrose consumption. In addition, the expression of Per1 genes in the mNAcSh was significantly reduced in mice treated with CGS 21680 compared with the controls. **CONCLUSIONS:** Our results suggest that A2a in the mNAcSh negatively regulates alcohol consumption which is associated with a reduction in the Per1 gene.

Disclosures: M. Parikh: None. **R. Sharma:** None. **V. Mishra:** None. **A. Soni:** None. **M. Thakkar:** None. **P. Sahota:** None.

Poster

P738. Alcohol: Circuitry and Neurophysiology of Addiction I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P738.06

Topic: G.09. Drugs of Abuse and Addiction

Support: 5F31AA028167-03

Title: Neural dynamics of striatal dopamine projection predict individual alcohol drinking

Authors: *S. MONTGOMERY[1], C. MOREL[1], A. GODINO[2], L. LI[1], S. J. RUSSO[1], E. S. CALIPARI[3], E. J. NESTLER[4], M.-H. HAN[1];
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Abstract: Harmful alcohol use is an increasing public health issue, currently resulting in 3 million deaths globally and contributing to more than 200 disease and injury conditions per year. An interesting phenomenon is the variability of alcohol consumption occurring within the population; some individuals drink casually while others drink in an uncontrolled or excessive manner. It is well established that the ventral tegmental area to nucleus accumbens (VTA-NAc) striatal dopamine projection is critical to encoding the primary reinforcing properties of both naturalistic stimuli and drugs. However, the mechanisms underlying how the neural dynamics of this circuit drive harmful alcohol use in some individuals and not others, remain vague. Using cell- and circuit-specific *in vivo* calcium imaging (fiber photometry) and *in vivo* electrophysiology, we investigated neural and behavioral signatures that predict voluntary alcohol drinking in mice and performed longitudinal assessments of how these phenotypes diverge or converge with continued alcohol consumption. We found that innate differences in reward sensitivity and exploratory behavior predict future alcohol phenotype. Conversely, we found that high levels of voluntary alcohol consumption normalize behavioral traits that are present in mice prior to alcohol exposure. In tandem, we show that VTA-NAc neural dynamics at baseline predict the future development of alcohol drinking phenotype, and these dynamics elucidate different mechanisms and consequences during encoding of naturalistic stimuli. Using *in vivo* electrophysiology, we determined that a higher pattern of VTA DA neuronal activity is associated with lower consumption of alcohol in the future, suggesting an innate protective effect of this circuit activity. Lastly, using RNA-sequencing, we investigated how the transcriptional landscape within the VTA and NAc shape distinct alcohol drinking profiles, aiming to identify molecular targets for future therapeutic use. By assessing the neural dynamics of this projection and naturalistic mammalian behaviors prior to and after voluntary alcohol drinking, this project will provide novel insights into the neural and behavioral risk signatures for the future development of alcohol misuse, as well as uncover novel mechanisms harnessed by individuals that are resilient to excessive alcohol drinking.

Disclosures: S. Montgomery: None. **C. Morel:** None. **A. Godino:** None. **L. Li:** None. **S.J. Russo:** None. **E.S. Calipari:** None. **E.J. Nestler:** None. **M. Han:** None.

Poster

P738. Alcohol: Circuitry and Neurophysiology of Addiction I

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Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P738.07

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant AA020919
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Title: Peripheral-central signaling involving dopamine 2 receptors affects locomotor activity and dopamine dynamics within the mesolimbic dopamine circuit

Authors: *S. C. STEFFENSEN[1], J. D. OBRAY[2], C. SMALL[1], J. LEBARON[1], E. BALDWIN[1], E. JANG[4], J. T. YORGASON[3];
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Abstract: Recent research has demonstrated that peripherally administered dopamine (DA) enhances DA levels in the nucleus accumbens (NAc). This enhancement of DA levels is accompanied by changes in locomotor activity and a reduction in negative affect associated ultrasonic vocalizations (USVs) and fear responses. These changes have been posited to occur through a mechanism involving increased firing of VTA DA neurons. Despite this, the mechanism underlying this effect is poorly understood. In this study we demonstrated that administration of domperidone, a peripheral dopamine 2 receptor (D2R) antagonist, prior to administration of peripheral DA attenuated the effects of peripheral DA on DA levels in the NAc. Further, single unit recordings of ventral tegmental area (VTA) DA neurons demonstrated that VTA DA neurons display a biphasic response to peripherally administered DA characterized by a brisk inhibition followed by a longer period of excitation. The excitation portion of the response of VTA DA neurons to peripheral DA was blocked by pretreatment with domperidone. Further, it was found that peripherally administered DA was mildly rewarding in a place conditioning paradigm, an effect which was blocked by domperidone. Many drugs of abuse are known to enhance sympathetic tone, resulting in increased circulating DA resulting from DA spillover at sympathetic nerve terminals. This observation suggests that some effects of drugs of abuse such as ethanol on DA neurotransmission may be mediated in part by activation of peripheral D2Rs. This hypothesis was tested by measuring DA levels in the NAc following ethanol administration in rats pretreated with vehicle or domperidone. It was observed that domperidone reduced ethanol mediated increases in NAc DA levels, consistent with the hypothesis that a portion of the effect of ethanol on DA neurotransmission would be mediated by

peripheral D2Rs. Further effects of pretreatment with domperidone were observed on ethanol induced sedation without any effect on ethanol reward or the amount of dopamine release from DA terminals in the NAc following phasic stimulation. These results are broadly consistent with a role for peripheral D2Rs in ethanol effects on the mesolimbic DA system.

Disclosures: S.C. Steffensen: None. **J.D. Obray:** None. **C. Small:** None. **J. LeBaron:** None. **E. Baldwin:** None. **E. Jang:** None. **J.T. Yorgason:** None.

Poster

P738. Alcohol: Circuitry and Neurophysiology of Addiction I

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Program #/Poster #: P738.08

Topic: G.09. Drugs of Abuse and Addiction

Support: K99AA025991
ZIA-AA000421
Fi2GM117604

Title: Striatal dopamine system mediates anxiolytic effects of ethanol

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Abstract: The literature suggests that anxiety is causally related to alcohol craving, consumption, and the development of alcohol use disorder (AUD). Alcohol has known anxiolytic properties, suggesting that individuals consume alcohol as a way to self-medicate against an aversive state. Animal models are consistent with this clinical observation: rats bred to consume large amounts of alcohol show higher basal anxiety and higher anxiolytic effects of alcohol compared to controls. Despite these behavioral observations, the underlying neural circuitry is unknown. The mesolimbic dopamine system has been implicated in drug abuse, including AUD. In both humans and rodents, alcohol abuse is associated with low levels of striatal dopamine D2 receptors (D2Rs). The current study implements transgenic mice with a targeted deletion of the D2R on striatal medium spiny neurons (MSNs) in order to better understand the neurobiology underlying the anxiolytic effects of alcohol. Mice with a knockout or knockdown of D2Rs on MSNs show enhanced anxiety-like behavior on the elevated zero maze, open field maze and light-dark box compared to littermate controls. They also show physiological indications of anxiety, such as elevated basal serum corticosterone levels. Further, the knockout and knockdown mice also show decreased preference for 1% sucrose compared to littermate controls, indicating anhedonia. When administered ethanol (1.2 g/kg; i.p.) these mice show enhanced anxiolytic effects of alcohol as measured on behavioral tasks. Interestingly, mice with

low levels of D2Rs on MSNs show increased activity of dopamine D1 receptor containing neurons, which leads to enhances anxiolytic effects of ethanol. Taken together, these data begin to describe the neural circuitry underlying and perpetuating the anxiolytic effects of alcohol.

Disclosures: M. Bocarsly: None. **V.A. Alvarez:** None.

Poster

P738. Alcohol: Circuitry and Neurophysiology of Addiction I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P738.09

Topic: G.09. Drugs of Abuse and Addiction

Title: Peripheral mechanoreceptor activation alters chronic ethanol-induced changes to VTA GABA neurons, NAc DA release and measures of withdrawal in rats

Authors: *K. BILLS[1,2], C. SMALL[2], D. OTTESON[2], G. JONES[2], J. BATES[2], S. STEFFENSEN[2];
[1]Noorda Col. of Osteo. Med., Springville, UT; [2]Psychology and Neurosci., Brigham Young Univ., Provo, UT

Abstract: There is growing evidence that mechanical stimulation (MStim) modalities (osteopathic and chiropractic manipulation, acupuncture and physical therapy) modulate substrates in the supraspinal central nervous system (CNS) that are outside the canonical somatosensory circuits. The aim of this study was to evaluate the effects of MStim applied to the cervical spine on neurons and neurotransmitter release in the mesolimbic dopamine (DA) system, an area implicated in reward and motivation. Utilizing electrophysiological, pharmacological and neurochemical techniques, in male Wistar rats, we demonstrate that low frequency (45-80 Hz), but not higher frequency (115 Hz), peripheral MStim to the lower cervical spine depresses firing of ventral tegmental area (VTA) GABA neurons (52.8% baseline; 450 sec) and increases VTA DA firing (248% baseline; 500 sec) and basal (178.43 % peak increase at 60 min) and evoked DA release in the nucleus accumbens (NAc; 135.03 % peak increase at 40 min). Furthermore, MStim-induced DA release was mediated, in part, by endogenous opioid and acetylcholine release in the NAc. Additionally, peripheral MStim, administered concurrently with alcohol, alters alcohol-induced desensitization of VTA GABA neuron firing rate in response to a reinstatement dose of ethanol (2.5mg/kg;IP) from 117.5 % of baseline to 32.3 %. Dopamine release in the NAc at 20 min post-injection was changed from 95.4% of baseline to 144.4 % and at 80 min from 104.1% to 138.2%. Further, behavioral indices of withdrawal (rearing, open-field crosses, tail stiffness and gait) were substantively ameliorated with concurrent MStim treatment.

Disclosures: K. Bills: None. **C. Small:** None. **D. Otteson:** None. **G. Jones:** None. **J. Bates:** None. **S. Steffensen:** None.

Poster

P743. Molecular and Neural Mechanisms I

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Program #/Poster #: P743.01

Topic: G.09. Drugs of Abuse and Addiction

Title: Low doses of psychostimulant and opiate co-administration augment short-term behavioral activation and acute withdrawal via D1 receptor activation

Authors: *I. WHITE, W. WHITE;
Morehead State Univ., Morehead, KY

Abstract: Previously, we reported that a high dose of either amphetamine (2 mg/kg) or morphine (5 mg/kg) reduces activity 12 to 24 hours after administration, a sign of acute withdrawal. Such reduction was prevented by SCH23390, a D1 antagonist, given 30 minutes after amphetamine or morphine. This study compared the effects of separate administration with co-administration of low doses of amphetamine and morphine on behavioral activation during the first several hours after administration (short-term effect) and on acute withdrawal. Male Wistar rats received a series of tests during which locomotor activity was monitored in an openfield for 24 hours after each treatment. Near light onset of Test Day 1, each animal received saline. On Test Day 4, each animal received amphetamine (0.5 mg or 1 mg/kg), morphine (1.25 mg or 2.5 mg/kg), a combination of amphetamine (0.5 mg/kg) and morphine (1.25 mg/kg), or the same combination followed 15 minutes later by administration of SCH23390 (0.05 mg/kg) to test reversibility by D1 receptor blockade. The combination of amphetamine (0.5 mg) and morphine (1.25 mg) produced dramatically more short-term behavioral activation and more intense and prolonged acute withdrawal, compared to doses given separately. SCH23390 prevented both the short-term activation and the acute withdrawal due to co-administered amphetamine and morphine, suggesting that the augmented effect is dopamine dependent. Recreational drug users self-administer combinations of stimulants and opioid substances (“speedballs”) to produce augmented drug effects, but such combinations may lead to greater health risks, such as increased intensity and duration of acute withdrawal, as well as overdose.

Disclosures: I. White: None. **W. White:** None.

Poster

P743. Molecular and Neural Mechanisms I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P743.02

Topic: G.09. Drugs of Abuse and Addiction

Title: Antagonizing serotonin 2A (5-HT_{2A}) receptors attenuates expression of methamphetamine-induced conditioned place preference in female but not male rats

Authors: *N. C. REYNA, J. T. MADDEN, K. B. COX, N. PENTKOWSKI;
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Abstract: Antagonizing serotonin 2A (5-HT_{2A}) receptors attenuates expression of methamphetamine-induced conditioned place preference in female but not male rats
Despite the need, there are currently no FDA-approved pharmacological treatments for methamphetamine abuse disorders. Cue-induced craving during drug withdrawal remains a significant contributor to high relapse rates. Thus, understanding the neurobiological mechanisms of cue-induced drug seeking will aid in the development of effective pharmacotherapies. Previous research has shown that the selective 5-HT_{2A} antagonist M100907 attenuates the acquisition of methamphetamine-induced conditioned place preference (CPP) in male and female adult rats. However, these findings have not been extended to blocking cue-induced drug-seeking behavior. Therefore, the present study examined the effects of M100907 on expression methamphetamine-induced drug-seeking behavior using a biased CPP design in adult male and female Long Evans rats (PND 90-111). During conditioning trials, rats were administered either saline or methamphetamine (1 mg/kg, i.p.) and then were immediately placed into their initially non-preferred chamber for 30 minutes. After four days of conditioning, rats either received vehicle or M100907 (0.0025, 0.025, 0.1 mg/kg i.p.) 15 minutes prior to expression testing. Pretreatment with the high dose of M100907 (0.1 mg/kg) attenuated methamphetamine-induced expression of CPP in females. In contrast, none of the doses affected expression in males. These data suggest that blocking the 5-HT_{2A} receptor can decrease cue-induced methamphetamine-seeking behaviors, and provide a promising target for treating methamphetamine use disorder.

Disclosures: N.C. Reyna: None. **J.T. Madden:** None. **K.B. Cox:** None. **N. Pentkowski:** None.

Poster

P743. Molecular and Neural Mechanisms I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P743.03

Topic: G.09. Drugs of Abuse and Addiction

Title: Antagonizing serotonin 2A (5-HT_{2A}) receptors with M100907 attenuates methamphetamine-induced reward in adult male and female rats and blocks methamphetamine-induced anxiety-like behaviors in adult male rats

Authors: *J. T. MADDEN, N. C. REYNA, E. V. GORANSON, N. S. PENTKOWSKI; Psychology, Univ. of New Mexico, Albuquerque, NM

Abstract: Antagonizing serotonin 2A (5-HT_{2A}) receptors with M100907 attenuates methamphetamine-induced reward in adult male and female rats and blocks methamphetamine-induced anxiety-like behaviors in adult male rats
John T. Madden[1], Nicole C. Reyna[1], Emerald V. Goranson[1], Nathan S. Pentkowski[1]
Department of Psychology[1], University of New Mexico, Albuquerque, New Mexico
Methamphetamine is a highly addictive and globally used psychostimulant. Currently, there are no FDA approved pharmacological treatments for methamphetamine use disorders, highlighting the need for research examining the neural mechanisms underlying the rewarding effects of methamphetamine and the factors that perpetuate sustained methamphetamine use. Research indicates that serotonin 2A (5-HT_{2A}) receptors play a key role in the mediation of psychostimulant-induced behaviors, including conditioned place preference (CPP). These findings have yet to be extended to methamphetamine. The present studies investigated the effects of the selective 5-HT_{2A} receptor antagonist M100907 on methamphetamine-induced CPP in adult male and female rats, and methamphetamine-induced anxiety-like behaviors in adult male rats. Adult male and female rats were tested for methamphetamine CPP using an unbiased two-chamber apparatus across eight consecutive days. During each conditioning session rats were pretreated with an assigned dose of M100907 (0, .0025, .025 or .25 mg/kg, i.p.) 15 minutes prior to methamphetamine administration (0 or 1 mg/kg, i.p.). Following methamphetamine administration, rats were immediately confined to their initially non-preferred chamber. After four conditioning sessions, the effects of M100907 on methamphetamine-induced shifts in CPP were assessed. Following the eight days of CPP testing, adult male rats were screened for anxiety-like behaviors in the elevated plus-maze. Pretreatment with M100907 attenuated methamphetamine-induced CPP in both male and female subjects, without producing any rewarding or aversive effects when administered alone. In male subjects, pretreatment with M100907 prevented methamphetamine-induced elevations in anxiety-like behavior. Additionally, M100907 attenuated some behavioral indices of anxiety in methamphetamine-naïve male rats. These findings add to a growing body of evidence suggesting that 5-HT_{2A} receptors may be an effective target for the treatment of methamphetamine use disorders.

Disclosures: J.T. Madden: None. **N.C. Reyna:** None. **E.V. Goranson:** None. **N.S. Pentkowski:** None.

Poster

P743. Molecular and Neural Mechanisms I

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Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P743.04

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH P20GM103423

Title: Sex, Drugs, and Choline: Interactive effects of biological sex and dietary choline on the locomotor response of rats to amphetamine

Authors: L. AUGUSTIN[1], E. DONIGAN[2], K. WALKER[3], A. SCHULMAN[3], &. ADEOYE-LAWAL[1], J. HERSH[1], *M. GLENN[1];
[1]Psychology, [2]Anthropology; Chem., [3]Biol., Colby Col., Waterville, ME

Abstract: Choline is an essential dietary nutrient integral to the development and proper function of the central nervous system. Among its many biological roles, it is the precursor to acetylcholine, an integral component of cell membranes, and the primary source of methyl groups for methylation reactions. In rats, the availability of choline during developmental sensitive periods exerts profound and persistent impacts on several neural systems. Notably, there are marked enhancements in cell signaling and neural plasticity, particularly in hippocampal-cortical circuits, with prenatal and adolescent choline supplementation and diminished function with choline deficiency. Aligning to these neural changes are a host of alterations to behavior. Adult rats whose mothers consume a choline-rich diet exhibit enhanced learning and memory and reduced stress reactivity and anxiety and these effects are resistant to age- and disease-associated declines. Choline deficient mothers produce offspring that are severely compromised under challenging cognitive conditions, inhibited, and anxious, with worse outcomes with age and disease. The aim of the present study was to extend these findings into the domain of addiction and reward with an initial focus on stimulant drugs that work mainly as dopamine agonists. Given the widespread action of choline in the nervous system and its effects on neural plastic mechanisms, we hypothesized that choline availability during adolescence would alter rats' responsivity to amphetamine and that these effects would vary as a function of biological sex. To test this hypothesis, female and male Sprague Dawley rats were fed synthetic diets (AIN76A) in adolescence: either 0, 1.1 or 5 g/kg of choline chloride (DEF, STD, and SUP, respectively). As adults, rats' locomotor responses to each of three doses of amphetamine (0, 0.5, and 1 mg/kg) was assessed using a modified Latin square design. Consistent with other research, there were marked sex differences in activity: females were more active than males overall and in response to amphetamine. There were also interactive effects of sex and diet: female SUP rats were more active than STD or DEF rats in response to 0.5 mg/kg amphetamine and DEF rats showed a prolonged response to that dose when compared to STD rats; these patterns were not observed in male rats. These findings indicate that adolescent choline supplementation may modify reward circuits to alter the way that stimulant drugs act there and may do so in a sex-specific way. Additional analyses are focused on how choline influences the rewarding properties of these drugs, rats' motivation to seek them out, and the neural bases for these changes.

Disclosures: L. Augustin: None. **E. Donigan:** None. **K. Walker:** None. **A. Schulman:** None. **&. Adeoye-Lawal:** None. **J. Hersh:** None. **M. Glenn:** None.

Poster

P743. Molecular and Neural Mechanisms I

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Program #/Poster #: P743.05

Topic: G.09. Drugs of Abuse and Addiction

Support: Grant from Colombo Plan Secretariat given directly to CINVESTAV.
Scholarship (No. 489884) from Conacyt, Mexico.

Title: Rewarding and relapse effects of levamisole and fentanyl as adulterants in methamphetamine.

Authors: *M. I. PAZ RAMOS[1], T. BROWNE, Jr[2], S. L. CRUZ[1];
[1]CINVESTAV, IPN, Mexico City, Mexico; [2]Colombo Plan Secretariat, Drug Advisory Program, Colombo, Sri Lanka

Abstract: Levamisole (LEV) is one of the most prevalent psychostimulant adulterants; nevertheless, its potential to induce relapse, alone or in combination, has not been assessed. Also, fentanyl (FEN) has been recently identified in various drugs, including methamphetamine (METH). Data on the opioid and stimulant combinations are well known, but information about FEN and METH co-administration is scarce. Here, we explored the rewarding effects of these substances, the combination of sub-effective doses, and their potential to produce relapse in the conditioned place preference (CPP) paradigm on its four different phases. We trained 81 adult male Wistar rats (200-250 g) in the CPP apparatus and administered the drugs s.c. First, we standardized the experimental conditions to reproduce previously reported data of the rewarding and relapse effects of METH on CPP. After four 1 mg/kg METH administrations, animals spent more time in the drug-paired chamber than in the initially preferred. Preference returned to basal conditions after extinction training. A challenge dose of 0.5 mg/kg METH reinstated CPP. With 1 mg/kg LEV animals showed drug-induced CPP after four doses, CPP disappeared after extinction training, and a challenge 0.5 mg/kg LVM dose did not reinstate CPP. Animals treated with FEN changed their preference after conditioning with four 30 µg/kg administrations, this preference disappeared after four drug-free sessions, and a challenge 15 µg/kg FEN dose reinstated CPP. These results suggest that LVM has rewarding effects, but to a lower extent than METH and FEN. To our knowledge, fentanyl reinstatement on CPP was not previously reported. Next, we did dose-response curves to determine the sub-effective METH, LEV, and FEN doses: 0.01 mg/kg, 0.1, and 0.3 µg/kg, respectively. These doses were used for co-administration experiments. LEV+MET potentiated the rewarding effects of individual drugs, but FEN+MET did not. Cross-reinstatement experiments showed that 0.01 mg/kg METH did not induce relapse, and 0.1 mg/kg LEV increased the time spent in the drug-paired chamber, but this data did not

reach statistical significance. Present results suggest that LEV might increase METH effects and play a role in relapse.

Disclosures: M.I. Paz Ramos: None. **T. Browne:** None. **S.L. Cruz:** None.

Poster

P743. Molecular and Neural Mechanisms I

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Program #/Poster #: P743.06

Topic: G.09. Drugs of Abuse and Addiction

Support: PAPIIT IN305420 (UNAM, Mexico)

Title: Intra-accumbal administration of CB1 agonist arachidonyl-2-chloroethylamide (ACEA) modulates the expression of methamphetamine-induced locomotor sensitization through participation of GABAB receptors

Authors: *F. MIRANDA-HERRERA, J. C. JIMENEZ, F. CORTES-SALAZAR, R. I. RUIZ-GARCIA, A. I. BARRIENTOS-NORIEGA;
Univ. Nacional Autonoma De Mexico, Tlanepantla, Edo Mex, Mexico

Abstract: INTRODUCTION: We have previously reported that systemic administration of GABAB receptor agonist baclofen (BCF) blocked the development and expression of AMPH-induced locomotor sensitization (LS) and reduced amphetamine-induced discriminative cue. Although these findings and those of other labs have indicated that GABAB agonists, such as BCF, may be useful in the treatment of drug abuse, at the present, the mechanisms underlying the effects of BCF on psychostimulant-related behaviors are not well known. Saigusa and his colleagues have reported that the administration of delta1-opioid receptor agonist DPDPE into the nucleus accumbens (nAcc) increased the DA efflux in the nAcc, and the activation of the GABAB receptors in the nAcc suppressed the effects of DPDPE on DA efflux into the nAcc. In line with these results, in the present study, we evaluated the effects of intra-accumbal administration of CB1 agonist arachidonyl-2-chloroethylamide (ACEA) on the expression of methamphetamine (METH)-induced LS and if these effects could be modulated by GABAB receptor agonist BCF. METHOD. Male Wistar rats were divided in four groups (n=6) and treated with METH or saline during days 3-7 (development of METH-induced LS). On day 10 (expression of METH sensitization test) rats were treated with drugs. Group S-S was treated with saline, group M-M was treated with METH, group M-AM was treated with ACEA and METH, group M-ABCFM was treated with ACEA, BCF and METH. METH was administered ip and ACEA and BCF were administered into the nAcc shell (AP 1 mm from bregma, ML ± 1.3 mm, DV -6.8 mm).The behavioral activity was recorded for 60 min on open-field cages during development and expression of METH-induced LS. RESULTS. The data showed that

administration of ACEA induced an increase in locomotor activity in rats during the expression phase. However, BCF administration in the expression phase produced a reduction of the effect of ACEA on METH-induced LS. CONCLUSION: These results are in line with previous studies and suggest that BCF could be acting on DAergic nerve endings and plays an inhibitory role in the regulation of DA release into the nAcc, essential for the development and expression of psychostimulant-induced LS.

Disclosures: F. Miranda-Herrera: None. **J.C. Jimenez:** None. **F. Cortes-Salazar:** None. **R.I. Ruiz-Garcia:** None. **A.I. Barrientos-Noriega:** None.

Poster

P743. Molecular and Neural Mechanisms I

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Program #/Poster #: P743.07

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R03DA050962
NARSAD Young Investigator Award Brain & Behavior Research Foundation award 28469

Title: Effects of Hunger on Drug response in Zebrafish

Authors: *P. BANSAL, D. AGGARWAL, E. E. JUNG;
Univ. of Illinois at Chicago, Chicago, IL

Abstract: Clinical and preclinical studies suggest that physiological state, such as hunger and satiety, can affect behavioral response to abused drugs Here we investigate the relationship between hunger and drug responses (neuronal and behavioral) in zebrafish larvae. To analyze neural activity and behavioral responses to the psychostimulant drugs, we have used 6 days post fertilization (dpf) Zebrafish (*Danio rerio)* larvae as an animal model and administered them D-amphetamine hemisulfate, a scheduled-II psychostimulant drug, to record direct physiological responses such as locomotion (n=18), tail flipping behavior (n=18), heart rate (n=12) and motor neuronal expression (n=8) in the spinal cord of the larvae *in vivo* linked to locomotor behavior. We performed these experiments in starved and satiated groups and responses were recorded pre (control_starved and control_fed) and post drug administration (drug_starved and drug_fed) for 40 minutes in the same subjects to obtain the baseline and observe the change in the activity. We observed that the drug_starved group showed 50% higher activity than control_starved group whereas activity of drug_fed group was 30% lower than control fed. We observed that base lines for control groups decreased, which can cause decreased activity even after the drug exposure in fed groups. Similar trend was observed in tail flipping activity, heart rate and motor neuronal

expression as well. These findings can call for further research to investigate the relationship between drug addiction related neural circuits and hunger mediating neurons.

Disclosures: P. Bansal: None. **D. Aggarwal:** None. **E.E. Jung:** None.

Poster

P743. Molecular and Neural Mechanisms I

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Program #/Poster #: P743.08

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R33DA041876

Title: Striatal spinophilin function and amphetamine sensitization

Authors: *D. S. WATKINS[1], C. W. MORRIS[3], B. MUÑOZ[2], A. J. BAUCUM II[4]; [1]Med. Neurosci., [2]Dept. of Psychiatry, Indiana Univ. Sch. of Med., Indianapolis, IN; [3]Med. Neurosci., Indiana Univ. Purdue Univ. at Indianapolis, Indianapolis, IN; [4]Biol., Indiana University-Purdue Univ. Indianapolis, Indianapolis, IN

Abstract: Proper synaptic transmission is critical for maintaining neuronal communication. There is increasing evidence that in many striatal pathological disease-states such as Obsessive Compulsive Disorder (OCD), Attention Deficit Disorder (ADD/ADHD), drug addiction, and others, synaptic transmission is perturbed. Furthermore, the principle striatal neurons, medium spiny neurons (MSNs), in many striatal-dependent disease-states exhibit differential perturbations in downstream signaling. Signal transduction pathways that are localized to the post-synaptic density (PSD) of MSNs regulate protein phosphorylation in a tightly controlled manner. Aberrant phosphorylation state dynamics within striatal MSNs are observed in myriad striatal pathological disease-states. While serine/threonine kinases obtain substrate specificity, in part, by phosphorylating specific consensus sites, serine/threonine phosphatases such as protein phosphatase 1 (PP1), are much more promiscuous. To obtain substrate selectivity, PP1 associates with targeting proteins. Spinophilin is the major targeting protein that binds PP1 as well as multiple other synaptic proteins within the striatum. Our lab has found that modulation of the striatal dopaminergic milieu, impacts the spinophilin protein interactome. However, spinophilin function under amphetamine-dependent signaling such as those observed following amphetamine-induced behavioral sensitization is less well characterized. Here, we begin to report the functional role of spinophilin in mediating behavioral changes associated with amphetamine-induced locomotor sensitization and delineate changes in spinophilin interactions that may mediate behavioral changes associated with amphetamine sensitization. To more specifically elucidate spinophilin's function, we have generated multiple transgenic animals that allow for cell-specific loss of spinophilin as well as interrogation of spinophilin protein

interactions. Our data suggest that spinophilin interactions are modulated in specific cell types following psychostimulant administration. Mechanisms regulating spinophilin's interactions in specific cell types as well as loss of spinophilin in specific cell types in modulating amphetamine-induced behavioral sensitization responses will be discussed.

Disclosures: D.S. Watkins: None. **C.W. Morris:** None. **B. Muñoz:** None. **A.J. Baucum II:** None.

Poster

P743. Molecular and Neural Mechanisms I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P743.09

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA DA035435
NIGMS P20GM113109

Title: Evaluation of the roles of differential rearing and sex on cue-induced amphetamine-seeking and astrocyte expression

Authors: *T. D. FORT, T. J. MOSER, M. C. AZUMA, M. E. CAIN;
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Abstract: Previous research demonstrated that environmental enrichment during adolescence engenders lower responding for drug reinforcement during self-administration and cue-induced relapse following drug abstinence. However, few studies examined whether enrichment is able to attenuate escalations in drug intake during extended-access amphetamine (AMP) self-administration (SA). The current study used 6-hr SA procedures to elucidate the effect that differential rearing (DR) may have on overall AMP self-administration, relapse following forced abstinence, and the extent to which relapse can be mitigated by treatment with the therapeutic *N-acetylcysteine* (NAC). Given that DR alters glutamate homeostasis, the impact of DR and NAC treatment on astrocyte expression was evaluated via immunohistochemical (IHC) staining of GFAP+ cells in the mPFC and NAcc. We hypothesized that enrichment would protect against escalation and would result in the greatest GFAP expression. Male and female Sprague-Dawley rats were raised in their respective environmental conditions for 30 days. Enriched (EC) rats were raised communally, given novel arrangements of toys, and handled daily. Isolated (IC) rats were single-housed in hanging cages, lacked novel objects, and were not handled during the rearing period. Standard (SC) rats were pair-housed, lacked novel objects, and were only handled once a week during cage changes. After rearing, all rats were implanted with an indwelling jugular catheter. Upon recovery, rats were placed into the operant chamber to undergo 12, 6-hr FR-1 sessions. Active lever presses resulted in an infusion of AMP (0.1 mg/kg/infusion) which

was followed by a time-out period. After 12 sessions, all animals were given daily treatments of NAC (100 mg/mL; ip) or vehicle during the abstinence period while remaining in their respective environments. After 14 days, all animals were placed back into the operant chambers for a 2-hr long relapse test in the absence of AMP, and brains were extracted immediately after for IHC staining of GFAP. Enrichment significantly lowered overall AMP SA as well as escalations in AMP intake during the 12 SA sessions. During the relapse test, enrichment also significantly attenuated cue-induced AMP seeking. Cue-seeking was significantly greater in females when compared to males. NAC failed to attenuate cue-induced responding during the relapse test in any environmental condition. Our ongoing IHC analyses to date suggest the ability of enrichment to attenuate escalating intake and relapse is not due to enrichment-induced changes in astrocyte expression.

Disclosures: T.D. Fort: None. **T.J. Moser:** None. **M.C. Azuma:** None. **M.E. Cain:** None.

Poster

P743. Molecular and Neural Mechanisms I

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Program #/Poster #: P743.10

Topic: G.09. Drugs of Abuse and Addiction

Support: National Institute of General Medical Sciences of the National Institutes of Health under linked Award Numbers RL5GM118969, TL4GM118971, and UL1GM118970
BUILD grant GM118969
UTEP Neuroplasticity Lab Endowment Fund

Title: A proposed mechanism for stimulant-induced behavioral sensitization involving the transporter VMAT

Authors: V. GARCIA[1], S. A. COLLINS[2], A. J. TELLEZ[2], B. N. BRITO[2], R. A. PEREZ[2], M. MIRANDA-ARANGO[3], *E. CASTAÑEDA[4];
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Abstract: Repeated exposure to psychostimulants produces sensitization, an enhanced behavioral and neurochemical sensitivity to both the drug and drug-related cues, that is thought to sustain addictive behavior. It was hypothesized that VMAT, the protein responsible for storage of dopamine (DA) into vesicles, may be functionally altered to redistribute intracellular DA between cytoplasmic vesicular pools based on demands for drug-mediated overflow versus release evoked by depolarizing electrical stimulation. Two behavioral tests of this hypothesis utilized rats that were pretreated with the VMAT blocker tetrabenazine (TBZ) before each daily

treatment of a 7-day sensitizing regimen of amphetamine (AMPH). Aim 1 tested whether TBZ pretreatment blocked sensitization of AMPH evoked rotational behavior in unilateral striatal DA depleted rats, a response primarily mediated through the reversal of DA Transporter function. Aim 2 tested whether TBZ pretreatment blocked sensitization of electrically evoked rotational behavior (ESRB) in rats receiving unilateral electrical stimulation of the medial forebrain bundle, a response mediated through exocytotic-like activation of DA release. After a 7-day incubation period following the sensitization protocol, rats were tested for expression of sensitization or its blockade by measuring AMPH-evoked rotational behavior (Aim 1) or ESRB (Aim 2). Within Subjects comparisons during the induction phase corroborate behavioral sensitization and acute effects of TBZ to block behavioral sensitization (Aim 1). Total turns were found to be an inadequate measure of behavioral sensitization in intact animals (Aim 2). On the challenge day testing for expression of sensitization, Between Subjects comparisons revealed behavioral sensitization of both AMPH-evoked rotational behavior (Aim 1) and ESRB (Aim 2). Most importantly, sensitization was blocked by TBZ pretreatment in both pharmacokinetic driven and exocytotic-like driven behaviors. It is concluded that VMAT may play a critical role in behavioral sensitization to stimulant drugs. This research is significant because a common mechanism (ie, VMAT) may play a role in behaviors evoked by different mechanisms involving drug-related responses and other behaviors mediated by exocytotic processes, such as drug-related discriminative stimuli. Future research must evaluate the effects of VMAT blockade during AMPH exposure upon DA overflow/exocytosis and investigate potential pharmacotherapeutic interventions that target VMAT function.

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Poster

P749. Cocaine: Reinforcement, Seeking, and Reinstatement III

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P749.01

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant K99 DA045764

Title: Distress tolerance and prelimbic activity predict cocaine-seeking in male, but not female rats

Authors: *T. M. MOSCHAK[1], T. J. SLOAND[2], R. M. CARELLI[3];
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Abstract: Distress tolerance (DT) is defined as the ability to persist in challenging goal-directed behavior in the face of stress, and individuals with low DT exhibit heightened drug-seeking behavior. However, no preclinical studies have examined the neurobiology underlying this phenomenon. To assess this, we used in vivo electrophysiology in Long Evans rats (n = 15 female, n = 20 male) during a DT task to record neural activity in the prelimbic cortex (PrL) and nucleus accumbens (NAc) core, two brain regions implicated in drug-seeking. For the DT task, rats were initially trained to press a lever within a brief window of time in order to receive a sucrose pellet. On the DT test day, this brief window of time was progressively shortened so that it became impossible for animals to obtain a correct response, and we assessed how long animals persistently responded in the task as our measure of DT. Following the test day, rats underwent two weeks of self-administration for either water/saline or cocaine (1 mg/kg/inf) for 6 hr/day. Animals then began a 1-month period of experimenter-imposed abstinence to induce heightened drug-seeking behavior (Grimm et al., 2001). On day 28 of abstinence we reassessed DT and neural activity, and on day 30 we assessed cocaine-seeking behavior. Males had significantly higher DT than females ($p = 0.009$). Additionally, male (but not female) rats with low DT after 28 days of abstinence had significantly heightened drug-seeking behavior (male: $p = 0.048$; female: $p = 0.624$). Furthermore, we found that a subset of PrL neurons tracked behavioral persistence during the DT task. Critically, task-induced activity in these neurons (but not those in the NAc core) negatively correlated with subsequent drug-seeking behavior in males ($p = 0.027$). These findings suggest that males that can maintain heightened PrL activity during stressful/challenging contexts are less likely to persistently seek out drug later. Conversely, DT may not play as large of a role in drug-seeking in females. In total, these data demonstrate an important role for the PrL in DT and link this neural activity and behavior to drug seeking.

Disclosures: T.M. Moschak: None. **T.J. Sloand:** None. **R.M. Carelli:** None.

Poster

P749. Cocaine: Reinforcement, Seeking, and Reinstatement III

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P749.02

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA R01-DA031734

Title: Cues associated with social defeat stress recruit CRF in the nucleus accumbens and invigorate drug-seeking following abstinence from cocaine

Authors: *M. LEONARD[1], K. A. MICZEK[1,2];
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Abstract: Cocaine-dependent individuals experience intense drug cravings in anticipation of imminent drug availability. Acute stressors can further exacerbate heightened states of arousal produced by drug-related stimuli and increase propensity to seek and consume drug. The present studies extend ongoing work examining the role of the stress peptide CRF in facilitating maladaptive arousal in the presence of drug-predictive stimuli. In rats, CRF enhances instrumental behavior directed towards gaining access to cocaine, in part through local control of DA transmission in the nucleus accumbens (NAc). Because CRF modulates motivational states in both appetitive and aversive contexts, we explored the extent to which an ecologically relevant stressor (social defeat) augments cocaine self-administration under a chained response sequence (ie seeking-taking), and whether NAc-CRF is recruited. On alternating days, male rats were exposed to acute social defeat in the presence of a compound stimulus (CS^+: peppermint odor+3kHz tone) or an empty cage (CS^-: almond odor+25kHz tone); 4 sessions each. Microdialysis conducted in the presence of the conditioned cues revealed that the stress-CS^+ produced a rapid increase in both CRF and DA in the NAc, which was temporally distinct from a delayed rise in CORT. Separate rats were trained to self-administer cocaine under a chained schedule of reinforcement (FI-FR) in order to dissociate *anticipatory* (drug-seeking) from *consummatory* (drug-taking) behavior. Completion of an FI (5m) produced access to continuous cocaine reinforcement (FR1) on a separate lever (5m). Accelerating responding across the FI was considered an index of arousal in anticipation of impending drug access - ostensibly, when craving is heightened. After a 7d drug-free (ABST) period, rats were reintroduced to the drug context to self-administer cocaine in the presence of either stress-paired (CS^+) or neutral cues (CS^-) in a counterbalanced manner. Compared to pre-ABST, responding during the FI was elevated under both conditions. Critically, CS^+ exposure produced significantly higher anticipatory responding for cocaine than the CS^- ($CS^+ > CS^- >$ pre-ABST), suggesting that stress cues augmented the effects of ABST alone. Cocaine intake was similar across conditions. These data support the idea that environmental stressors can amplify the impact of returning to a drug-context after an abstinence period, such to invigorate putative “drug-seeking” behavior. Anticipation of threat may engage common motivational salience circuits that generate arousal during cocaine expectation, whereas unconditioned aspects of drug-reward are differentially controlled.

Disclosures: M. Leonard: None. **K.A. Miczek:** None.

Poster

P749. Cocaine: Reinforcement, Seeking, and Reinstatement III

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Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P749.03

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA14328

Title: Differential plasticity in nucleus accumbens core and shell mGlu5 glutamate receptor following cocaine self-administration is both dose and withdrawal dependent.

Authors: *M. GHASEMZADEH, O. BURGOS, M. AL RUBAYE, D. SHARMA, M. MIRZAEI;
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Abstract: Prolonged high level cocaine abuse leads to severe drug addiction in humans. This pattern of drug abuse also leads to gradual increase in drug intake over time and produces exacerbated drug craving during periods of abstinence known as incubation of craving. We can model this pattern of drug abuse by using extended-access cocaine self-administration in rats (LgA, 6hr daily session). A major obstacle in the treatment of addiction has been the propensity to relapse, often mediated by drug-associated cues, even after prolonged period of abstinence from drug use. Recent work suggests that glutamatergic signaling has an important role in cocaine addiction and particularly in drug seeking and relapse. In particular, the group1 metabotropic mGlu1/5 receptors have been shown to be important for both drug taking and drug seeking; and therefore, they have been pursued as promising targets for therapeutic development. Male Sprague-Dawley rats were trained to self-administer cocaine (FR1; 1.0 mg/kg/200 µl/inf) during either 2-hr (ShA) or 6-hr sessions (LgA) for 14 days. Subsequently, animals remained in the home cage for 3, 10, or 60 days. Following abstinence period, rats were tested under context-primed extinction condition for cocaine seeking after systemic administration of either saline or an mGlu1/5 receptor antagonist (JNJ16259685 or MTEP). Following a short abstinence period (3 or 10 days), the systemic blockade of mGlu5 receptor reduced drug seeking only in ShA subjects without affecting the LgA animals, while mGlu1 receptor blockade was effective in reducing drug seeking in both groups. However, after a long abstinence period (60 days), the systemic blockade of either receptor significantly reduced drug seeking in both ShA and LgA rats. It has been demonstrated that intracerebral infusion of MTEP (3µg/side) after 10 days of abstinence into either NAcore or NAshell leads to a decrease in drug seeking in ShA rats, whereas blockade of NAshell mGluR1 does not block drug seeking. However, our results in LgA rats suggest that while blockade of mGluR5 in NAcore reduced drug seeking, its blockade in NAshell was not effective in reducing drug seeking. These results suggest that exposure to cocaine produce a transient intake dependent plasticity in mGluR5, but not in mGluR1, function in the brain. Moreover, our data point to nucleus accumbens shell as the anatomical substrate contributing to the selective modulation of mGluR5 function in LgA rats. Our observations suggest that systemic activation of mGluR5 (CDPPB, 10 mg/kg), during early abstinent period may prevent development of incubation craving. Interestingly, this effect of mGluR5 PAM may emanate from NAshell.

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Poster

P749. Cocaine: Reinforcement, Seeking, and Reinstatement III

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Program #/Poster #: P749.04

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH grant R21 DA041755

Title: Renewal of cocaine seeking using social and nonsocial contexts

Authors: *B. A. HUMBURG, L. R. HAMMERSLAG, S. G. MALONE, P. S. KELLER, M. T. BARDO;
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Abstract: Individuals with substance use disorders undergo craving when re-exposed to a drug-associated context. It has been shown that social peers throughout abstinence can be protective; however, drug-associated peers can also serve as cues that trigger craving and relapse. To date, a majority of preclinical research assessing the mechanisms of relapse have been restricted to nonsocial cues or contexts (e.g., tones, lights or odors). The purpose of this study was to determine if cocaine seeking is differentially renewed by a social context (peer) vs. a non-social context (house light (HL) illumination). A dual-compartment apparatus was used in which rats could have limited social contact through a wire screen partition. In experiment 1, young adult Sprague-Dawley (7 male and 8 female) rats were trained with a 2-lever procedure to self-administer (SA) cocaine (0.75 mg/kg/inf) for 21 days in a context consisting of both a same-sex, same-age peer (non-self-administering) and illumination of a HL in each compartment (Context A). Half the rats were then extinguished in Context A and the other half in Context B (no peer or HL illumination). Renewal of cocaine seeking was then tested in the presence of Context A stimuli in counterbalanced order as follows: (1) peer alone; (2) HL alone; or (3) peer + HL compound. We also included a control test which involved the same context as extinction for each group. Multi-level modeling was used to analyze differences in responding between AAA and ABA groups. Following acquisition of cocaine SA, the AAA group extinguished at a faster rate than the ABA group. During renewal tests, the ABA group showed significant renewal to the peer alone and peer + HL compound when compared to extinction, whereas the AAA group did not. The HL alone did not renew cocaine seeking in either group, indicating that it was overshadowed completely by the peer. To ensure that the HL alone was salient enough to elicit renewal, Experiment 2 was conducted exactly the same as above (7 male and 8 female Sprague-Dawley rats) except that Context A consisted of only illumination of a HL in each compartment (no peer) and context B had no HL illumination. Again, following acquisition of cocaine SA, rats significantly decreased their responding, with the ABA group having more responding on the active lever than the AAA group. The renewal test showed that the ABA group increased responding to the HL illumination compared to extinction, whereas the AAA group did not. Overall, these results indicate that social peers can serve as powerful stimuli that can overshadow nonsocial stimuli in renewal of drug seeking. Supported by NIH grant R21 DA041755.

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Poster

P749. Cocaine: Reinforcement, Seeking, and Reinstatement III

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA [DA047976] (MV)
NARSAD Distinguished Investigator Grant Award (YS)

Title: The protective effect of operant social reward on cocaine self-administration, choice, and relapse is dependent on delay and effort for the social reward

Authors: *M. VENNIRO[1], L. V. PANLILIO[2], D. H. EPSTEIN[2], Y. SHAHAM[2];
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Abstract: Background: Social-reinforcement-based treatments are effective for many, but not all, people with addictions to drugs. We recently developed an operant rat model that mimics features of one such treatment, the community-reinforcement approach. In this model, rats uniformly choose social interaction over methamphetamine or heroin. Abstinence induced by social preference protects against the incubation of drug seeking that would emerge during forced abstinence. Here, we determined whether these findings generalize to cocaine and whether delaying or increasing effort for social interaction could reveal possibly human-relevant individual differences in responsiveness. Methods: We trained male and female rats for social self-administration (6 days) and then for cocaine self-administration, initially for 2-h/day for 4 days, and then for 12-h/day continuously or intermittently for 8 days. We assessed relapse to cocaine seeking after 1 and 15 days. Between tests, the rats underwent either forced abstinence or social-choice-induced abstinence. After establishing stable social preference, we manipulated the delay for both rewards or for social reward alone, or the response requirements (effort) for social reward. Results: Independent of cocaine-access conditions and sex, operant social interaction inhibited cocaine self-administration and prevented incubation of cocaine seeking. Preference for social access was decreased by delay of both rewards or social reward alone, or by increased response requirements for social reward, with notable individual variability. Conclusion: This choice procedure can identify mechanisms of individual differences in an animal model of cocaine use and could thereby help screen medications for people who are relatively unresponsive to treatments based on rewarding social interaction.

Disclosures: M. Venniro: None. **L.V. Panlilio:** None. **D.H. Epstein:** None. **Y. Shaham:** None.

Poster

P749. Cocaine: Reinforcement, Seeking, and Reinstatement III

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P749.06

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA P50 DA037844

Title: Genome-wide association study in outbred heterogeneous stock rats for the conditioned and unconditioned effects of cocaine

Authors: *E. RAKOWSKI[1], C. P. KING[2], A. S. CHITRE[3], O. POLESSKAYA[4], S. B. FLAGEL[5], T. E. ROBINSON[6], L. C. SOLBERG WOODS[7], A. A. PALMER[8], P. MEYER[9]; [1]Psychology, State Univ. of New York, Univ. at Buffalo, Buffalo, NY; [2]Psychology, Univ. at Buffalo, Buffalo, NY; [3]Dept. of Psychiatry, [4]Univ. of California San Diego, La Jolla, CA; [5]Mol. and Behavioral Neurosci. Inst., Univ. of Michigan, Ann Arbor, MI; [6]Dept Psychol, Univ. of Michigan Dept. of Psychology, Ann Arbor, MI; [7]Wake Forest Sch. of Med., Winston-Salem, NC; [8]Psychiatry, UCSD, La Jolla, CA; [9]Psychology, Univ. At Buffalo, Buffalo, NY

Abstract: Addiction vulnerability is frequently modelled in rodents using behavioral tasks that measure the conditioned and unconditioned effects of cocaine, both of which are shaped by genetic and environmental factors. To examine these heritable genetic factors, we performed a genome-wide association study (GWAS) to identify genomic regions associated with cocaine sensitivity in two tasks. Specifically, we characterized genotypically and phenotypically diverse heterogeneous stock (HS) rats in two cohorts (total n = 3,228) in cocaine conditioned cue preference (CCP) and contextual conditioning (CCC) tasks, in which tactile floors and contexts were paired with repeated cocaine treatment. We then conducted GWAS on measures of locomotor activity and conditioned responses to the cues and context. Measures of cocaine conditioning showing modest heritability (h^2 = .065-.255), and we found two loci on chromosomes 16 and 19 which were associated with the initial locomotor response to cocaine and approach to the cocaine-paired floor following conditioning, respectively. Each loci contained multiple genes (*e.g. Hmox1, Large1*). In contrast, most genetic loci we found reflected baseline locomotor behaviors and showed larger heritability values (h^2 = .308-.121). For example, locomotor activity during habituation sessions or saline treatments were associated with loci on chromosomes 1, 2, 6, 15, and 18, which included multiple genes (*e.g. Ptpn2, Spire1*). We further examined the 3Mb region surrounding each of these loci with other behavioral measures collected as part of this project using a phenome-wide association study (PheWAS), and found that these regions were also associated with other behaviors reflecting general exploratory behavior and arousal, including responding during light reinforcement and distance traveled in an open field test. These data indicate that the conditioned and unconditioned effects of cocaine, while influenced by genetic and heritability factors, are not largely informed by individual single genetic loci. Additional approaches using gene network analysis may be more useful for disentangling complex multi-gene system interactions underlying heritability. We found that more loci were detected for non-drug measures related to baseline exploratory

behavior and arousal. This study suggests that HS rats are useful for identifying gene systems influencing complex behaviors related to addiction vulnerability, although the utility of identifying individual loci may be dependent on the task.

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Poster

P749. Cocaine: Reinforcement, Seeking, and Reinstatement III

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Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P749.07

Topic: G.09. Drugs of Abuse and Addiction

Support: 5 F31 DA049505-03

Title: The experience of control mitigates stress-induced behavioral changes: Implications for cocaine demand and seeking

Authors: *K. A. SILETTI, K. T. BROWN, G. C. KNOPP, M. C. HILLMAN, E. C. WARNOCK, M. V. BARATTA, M. P. SADDORIS;
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Abstract: Motivation to obtain natural and drug rewards is sensitive to costs that an animal must endure to obtain them, such as risk and effort. Demand that is highly elastic will decay quickly as costs accrue, while inelastic demand will persist despite escalating costs. Following chronic, voluntary drug administration, drugs often serve as inelastic reinforcers; animals will often persist drug-seeking despite decreasing dose or drug availability. However, this demand can be further modulated by factors such as exposure to stress or drug-associated cues. Specifically, inescapable stress or re-exposure to drug paraphernalia can induce intense drug cravings and promote relapse, even during prolonged abstinence.
Our work explores how prior experience with stress modulates cost sensitivity and motivation to seek sucrose and cocaine reward. While stress is an unavoidable aspect of life, not all stressors are inescapable. Learning to control stress can alternatively have protective benefits against future stressors. Animals that are trained to control stressors do not demonstrate enhanced hypervigilance or neophobia otherwise exhibited by uncontrollable stress-exposed counterparts. In the “Escapable Stress/Inescapable Stress”, or ES/IS, model of stressor controllability, an “ES” rat can turn a wheel to terminate a 1 mA tail-shock. A yoked “IS” rat receives the exact same intensity and duration of shock, without the experience of control. Our work demonstrates that ES-experienced animals are less sensitive to effort costs when the reward is sucrose, as ES-experienced animals will lift weight similar to no stress controls. However, using a behavioral

economics paradigm in which cocaine cost is manipulated by decreasing the dose delivered per infusion, we demonstrate that these same stress experiences may have no effect on cost sensitivity when the outcome is cocaine.
Moreover, we show preliminary data suggesting that stressor controllability training may reduce drug-seeking in comparison to IS-experienced controls, but only during abstinence when the drug is unavailable. Animals that experience ES did not incubate to cocaine-associated cues over 30 days of forced abstinence, while IS-experienced animals exhibited greater drug-seeking on withdrawal day 30 than day 1. Our work suggests that stressor controllability may moderate aspects of cocaine-seeking under abstinent conditions wherein drug is unavailable; alternatively, when drug is available, demand for cocaine is insensitive to benefits of stressor controllability training.

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Poster

P749. Cocaine: Reinforcement, Seeking, and Reinstatement III

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P749.08

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH/NIDA Grant DA046457

Title: Schedules of reinforcement influence cocaine-seeking patterns that correspond with goal-directed and habitual behavior in rats

Authors: *S. N. HANDEL[1], B. O. JONES[2], R. J. SMITH[3];
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Abstract: Our lab recently developed a novel method to discriminate goal-directed and habitual cocaine seeking in rats using outcome devaluation via satiety. Sensitivity to outcome devaluation indicates the use of a goal-directed strategy, whereas insensitivity indicates the use of a habitual strategy. We found that random ratio (RR) and random interval (RI) schedules of reinforcement bias responding to be goal-directed or habitual, respectively, but that cocaine seeking can be

goal-directed or habitual on both schedules. Here, we wanted to further explore how response strategies and reinforcement schedules interact. We trained male Sprague Dawley rats to self-administer intravenous cocaine on a seeking-taking schedule of reinforcement with RR20 or RI60 requirements on the seeking lever. To assess response strategy, the cocaine outcome was devalued via satiety by administering noncontingent intravenous cocaine prior to a 10-min extinction test. Results thus far indicate that the occurrence of goal-directed or habitual strategies within a schedule corresponds with distinct behavioral patterns that are particular to that schedule. As compared to goal-directed responding, habitual responding is associated with increased inter-infusion variance on the RR schedule but decreased variance on the RI schedule. Further analyses indicate that the schedule also influences strategy-specific patterns of responding on the seeking lever, including number of responses and rate of responding. These behavioral analyses will provide greater insight into how schedule influences response strategy, as well as how response strategy influences patterns of cocaine seeking and taking. Understanding the interactions between schedule and response strategy will be key to developing better animal models of habitual and compulsive drug seeking, and to unraveling the behavioral and neural mechanisms of habits in addiction.

Disclosures: S.N. Handel: None. **B.O. Jones:** None. **R.J. Smith:** None.

Poster

P749. Cocaine: Reinforcement, Seeking, and Reinstatement III

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P749.09

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH grant AA023305

Title: Predator odor stress exposure produces sex-specific effects on cocaine self-administration in rats

Authors: *T. J. TEMPLETON, N. W. GILPIN;
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Abstract: Post traumatic stress disorder (PTSD) and cocaine use disorder (CUD) are highly comorbid and pose serious health consequences. Women are twice as likely to develop PTSD than males; and females with CUD are more likely to have PTSD than males with CUD. Despite these clinical findings, preclinical research investigating the role of stress on cocaine use has been conducted exclusively in males. This project was designed to 1) identify the effects of traumatic stress exposure on operant cocaine self-administration, and 2) identify neurochemical changes following stress-induced cocaine self-administration in males and females. Male and female Wistar rats were trained to self-administer cocaine intravenously for 10 days, then were

exposed to predator odor (i.e., bobcat urine) and tested for avoidance of the odor-paired context. Unstressed controls were not exposed to predator odor. Animals then once again self-administered cocaine for 10 days either 1hr/day (short access [ShA]) or 6hr/day (long access [LgA]) before tissue was collected for analysis. Preliminary results suggest that predator odor exposure promotes escalation of cocaine self-administration in female (but not male) rats with long access but not short access to cocaine. Stressed LgA females exhibited escalation of cocaine intake across 10 days of self-administration following predator odor stress exposure relative to 1) unstressed LgA females, 2) stressed ShA females, and 3) stressed LgA males. Additionally, results suggest that females (but not males) with long access to cocaine gain less weight post-stress than unstressed controls and females with short access to cocaine. These findings suggest that females are at a higher risk of developing cocaine use disorder following stress exposure than stressed males and unstressed females. Our data also suggest that stressed male rats exhibit blunted escalation, or perhaps even reductions, of cocaine intake relative to unstressed male controls. Ongoing work is measuring stress peptide content in the brains of these animals. This work was funded in part by NIH grant AA023305.

Disclosures: T.J. Templeton: None. **N.W. Gilpin:** F. Consulting Fees (e.g., advisory boards); Glauser Life Sciences, Inc..

Poster

P749. Cocaine: Reinforcement, Seeking, and Reinstatement III

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P749.10

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA039865
NIH Grant DA046117

Title: Prenatal circadian rhythm disruption induces sex-specific substance use-related phenotypes in mice

Authors: *L. DEPOY[1], C. A. MCCLUNG[2];
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Abstract: 20% of Americans are at risk for environmental circadian rhythm disruptions (CRD) due to shift work. These individuals experience substantial negative health outcomes, but females are especially affected with greater vulnerability for substance use (SU) and adverse outcomes associated with pregnancy. These outcomes not only occur *during* pregnancy, but offspring are also affected at birth and later in life. Prenatal CRD (pCRD) in mice recapitulates these risks, increasing adverse pregnancy outcomes and altering behavior in adult offspring.

However, it is unknown whether pCRD affects SU in mature offspring. C57Bl/6J dams were sham handled or disrupted, by reversing the light/dark cycle 4 times during gestation. Reward-related behaviors were measured in mature offspring. Cocaine reward was measured using conditioned place preference (5 mg/kg cocaine). Contingency degradation was used to measure decision making. Mice were trained to respond on two levers for food, then the likelihood that one of those levers will be reinforced was degraded. Another cohort was trained to respond for food before jugular catheterization. After recovery, mice were trained to respond on another lever for cocaine. Acquisition, the reinforcing and motivational properties of cocaine, extinction and cue-induced reinstatement were measured. Interestingly, females exposed to pCRD developed an anhedonic-like phenotype with decreased food self-administration, cocaine intake and reinforcing properties of cocaine. On the other hand, males showed a SU-like phenotype with increased cocaine preference, higher order food self-administration and cocaine reinforcement. Furthermore, male pCRD mice maintained goal-directed decision making, responding more on a reinforced aperture, while female pCRD mice did not, indicating habit formation. Together these results suggest that male and female mice exposed to pCRD respond differently for rewarding outcomes. In order to determine whether these divergent behavioral outcomes are unique to reward I then measured anxiety-like behavior. As expected, behavior in the open field test and elevated plus maze paralleled reward-related behavior. Anhedonic-like female pCRD mice showed increased anxiety-like behavior and pCRD males showed decreased anxiety/increased risk-taking behavior. These results suggest that pCRD may predispose individuals to distinct psychiatric disorders based on sex, mood disorders in females and SU disorders in males. By understanding how disrupted rhythms during pregnancy affect behavior in adulthood, we can develop novel therapeutic approaches for SU and mood disorders in adults.

Disclosures: L. Depoy: None. **C.A. McClung:** None.

Poster

P749. Cocaine: Reinforcement, Seeking, and Reinstatement III

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P749.11

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA048280

Title: Aversive stimuli that decrease nucleus accumbens dopamine signaling also increase drug preference

Authors: E. M. GRAFELMAN[1], D. S. WHEELER[1], M. GELIN[1], P. LAMBERTON[1], M. C. HEARING[1], J. R. MANTSCH[2], *R. A. WHEELER[1];
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Abstract: Aversive stimuli have a complex impact on the neural systems responsible for the pursuit of drug and natural rewards. Aversive stimuli can punish reward-seeking behaviors, but in a different situation, the same stimuli can invigorate drug-seeking behaviors. Physiologically, aversive stimuli can acutely suppress dopamine (DA) release in the nucleus accumbens (NAc) but may simultaneously stimulate dopamine release in other brain areas, such as the prefrontal cortex (PFC). Here we investigated the effect of various aversive stimuli on DA signaling in the NAc and PFC using fiber photometry. Then we examined the effect of an aversive stimulus on the self-administration of freely available drug and natural rewards. Female and male rats underwent surgery in which they received an infusion of AAV5-hSyn-dLight 1.3 into the NAc or medial PFC and implantation of an optic fiber to the same location. Following recovery, the effects of different aversive stimuli on DA signaling in the NAc and PFC were examined. The aversive tastant, quinine, an intense white noise, and electric footshock were each found to reliably reduce DA in the NAc while increasing DA in the PFC. Following this determination, a separate group of rats was implanted with intrajugular catheters and tested in a free choice design. Rats were trained to press a lever for sucrose pellets and trained to press a lever for an intravenous infusion of cocaine followed by a 20s timeout. After training, rats were tested in a free choice design in which responding on one lever provided two sucrose pellets and responding on the other provided an infusion of cocaine. Both levers were available throughout the 120 min session. Following the establishment of stable responding for both the natural and drug rewards in daily sessions, an intense white noise aversive stimulus was presented during the task on alternating days. For both male and female rats, the presence of the white noise stimulus increased cocaine self-administration. Following extinction training on the cocaine lever, white noise reduced responding on both levers. These data show that an aversive stimulus can increase the motivation to seek a drug reward, and that decreased NAc DA signaling is a physiological correlate of this process. Ongoing studies are monitoring DA release in the NAc during the free choice task in the presence of the aversive stimulus.

Disclosures: E.M. Grafelman: None. **D.S. Wheeler:** None. **M. Gelin:** None. **P. Lamberton:** None. **M.C. Hearing:** None. **J.R. Mantsch:** None. **R.A. Wheeler:** None.

Poster

P749. Cocaine: Reinforcement, Seeking, and Reinstatement III

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Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P749.12

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH R01 DA046457

Title: Effects of punishment on goal-directed and habitual responding for food and cocaine.

Authors: *B. JONES[1], R. J. SMITH[2];
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Abstract: Addiction is characterized by compulsive drug use that persists even when faced with negativeconsequences. Animals that are resistant to punishment will continuing to self-administer cocaine despite receiving footshock. Our lab is interested in testing the hypothesis that punishment resistance arises from failure to exert goal-directed control over habitual drug seeking for intravenous (IV) cocaine. To directly test the relationship between punishment resistance and habits, our lab developed a novel outcome devaluation procedure for IV cocaine that can be used to discriminate goal-directed (GD) and from habitual (H) response strategies. Previous work from our lab has established a link between response strategy and punishment resistance for IV cocaine and food. We found that being GD or H prior to receiving punishment does not predict how animals will respond to punishment. In contrast, we find that sensitivity to punishment is associated with the response strategy utilized after punishment. Additionally, we find that punishment can cause rapid shifts in response strategy. A habitual rat that is sensitive to punishment can quickly shift to a GD strategy and a GD rat that is resistant to punishment can shift to a habitual strategy. However, we also find that some H rats remain habitual after punishment, and some GD rats will remain GD. Here, we investigate if the way a rat shifts response strategy (i.e. a rat shifting from GD to H) is correlated with punishment sensitivity and recovery from punishment. Male Sprague Dawley rats were trained on a seeking-taking chained schedule of self-administration for either food pellets or intravenous cocaine, in which completion of a random ratio or random interval schedule on the seeking lever gave access to a separate taking lever. Animals were then exposed to 4 days of punishment testing, in which footshock (0.4 mA, 0.3 s) was delivered randomly on one-third of trials, immediately following completion of seeking and prior to extension of the taking lever. Response strategy was assessed 4 days before and after punishment. While being H after punishment was correlated with resistance to punishment, rats that were GD prior to punishment and switched to a H strategy were more resistant to punishment than the H rats that did not switch. Similarly, rats that shifted from H to GD were more sensitive to punishment than rats that remained GD throughout testing. Interestingly, within the group of rats that remained GD, we found no correlation with sensitivity to footshock. Taken together, these data suggest that sensitivity to punishment utilizes GD behavior, however, a rat utilizing GD behavior is not necessarily sensitive to punishment.

Disclosures: B. Jones: None. **R.J. Smith:** None.

Poster

P749. Cocaine: Reinforcement, Seeking, and Reinstatement III

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Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P749.13

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH/NIDA grant R00DA039991

Title: Behavioral and neuroactivational correlates of psychosocial stress-induced cocaine seeking in rats

Authors: *N. M. HINDS, I. D. WOJTAS, D. M. PULLEY, S. J. MCDONALD, S. DE GUZMAN, N. E. HUBBARD, C. M. KULICK-SOPER, J. J. DEBSKI, B. PATEL, D. F. MANVICH;
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Abstract: A major challenge for treating cocaine use disorder is the high risk of relapse that can persist even after prolonged periods of abstinence. Exposure to psychosocial stress induces cocaine craving and is thought to be a major contributor to cocaine relapse, yet the neural circuitry by which it induces drug seeking has not been thoroughly explored. This study aimed to identify brain regions selectively activated during psychosocial stress-induced cocaine seeking in rats. Adult male and female Long-Evans rats were trained to self-administer cocaine (0.5 mg/kg/inf, i.v.) in 2-h daily operant-behavioral sessions for 20 d. On days 11, 14, 17, and 20, a discrete tactile cue was presented in the operant chamber and these sessions were immediately followed by social stress (social defeat, n=16), nonsocial stress (footshock, n=12), or a no-stress control condition (n=12). Beginning on day 21, animals underwent extinction training during which lever-presses were not reinforced. Once responding was extinguished, rats were re-exposed to the tactile cue that signaled their assigned stress/no-stress stimulus and cocaine seeking was measured for 2 h under extinction conditions. Immediately after this reinstatement test, animals were sacrificed, and brains collected and processed for c-Fos expression. When all groups were combined, cocaine-seeking magnitude was found to be positively correlated with neural activity in several brain areas, including the rostral periaqueductal gray (rPAG). Interestingly, activity within different subdomains of the rPAG varied as a function of the stressor employed, as activation of the dorsomedial rPAG correlated with footshock-associated cocaine seeking while activation of the dorsolateral and lateral rPAG correlated with psychosocial stress-associated cocaine seeking. Moreover, activity in these rPAG subdomains were differentially correlated with activation in other brain areas. Finally, ethographic analysis of social defeat episodes revealed that rPAG activity at the time of the cocaine seeking test was positively correlated with prior display of “active-defensive” coping behaviors in response to social threat. Taken together, our results suggest that psychosocial stress may trigger cocaine seeking via a unique neural network that involves the dorsolateral and/or lateral rPAG, and that the rPAG may represent a shared neural substrate mediating active coping responses to social stress as well as social stress-induced drug-seeking behavior. Studies are currently underway to examine the functional role of the rPAG in stress-induced cocaine seeking behavior.

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Poster

P897. Learning and Memory in Aging: Non-Human Primates and Humans

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P897.01

Topic: H.12. Aging and Development

Support: NIH P51OD010425
AHA-Allen Initiative in Brain Health and Cognitive Impairment: 19PABH134610000

Title: Performance on a visuo-spatial working memory task in adolescent and aged rhesus macaques

Authors: *M. MUNOZ[1], M. L. JUTRAS[1], C. GLAVIS-BLOOM[2], J. H. REYNOLDS[3], C. R. VANDERLIP[3], A. D. GARCIA[1], E. BUFFALO[1];
[1]Univ. of Washington, Lynnwood, WA; [2]Neurosci., Salk Inst. for Biol. Studies, La Jolla, CA; [3]SNL-R, Salk Inst., La Jolla, CA

Abstract: Results from a large and long-standing body of literature demonstrate that aged non-human primates (NHPs) are impaired on short-term visuo-spatial memory tasks compared to younger NHPs (Moss et al., 1997; Amaral & Rapp, 1989). One such task, the Delayed Recognition Span Task (DRST), has been employed in both NHP and human studies (Moss et al, 1989). In this task, the subject is rewarded for identifying a novel stimulus amongst a successively increasing number of familiar stimuli by remembering information about the stimuli's visual features, spatial locations, or a combination of both. Here, we trained adolescent (n=2, mean age=5 yo) and aged (n=3, mean age=20 yo) female rhesus macaques on a touch-screen version of visuo-spatial DRST using tablets mounted to their home cages (Lafayette Instrument Company, Lafayette, IN). To measure individual animal performance on the DRST we assessed memory capacity by calculating, on average, how many correct stimuli the monkey chose on each trial. All macaques, regardless of age, learned to perform this task above chance levels. However, performance differences were noted across age groups. Specifically, there was greater individual variability in maximum DRST performance amongst aged macaques compared to young (SD, young: 0.94; aged: 2.28), and aged macaques had a lower average span length compared to young (span length, young: 7.17; aged: 4.26). Taken together, these findings demonstrate the potential of home-cage automated testing and suggest that performance on the DRST is impaired in some aged macaques. This flexible testing paradigm will enable future studies with larger arrays, multiple delays, and a comparison of visual vs spatial memory. Further, this task could be used in longitudinal studies to identify the progression of age-related memory impairment in NHPs.

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Poster

P897. Learning and Memory in Aging: Non-Human Primates and Humans

Location: Virtual Only

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Program #/Poster #: P897.02

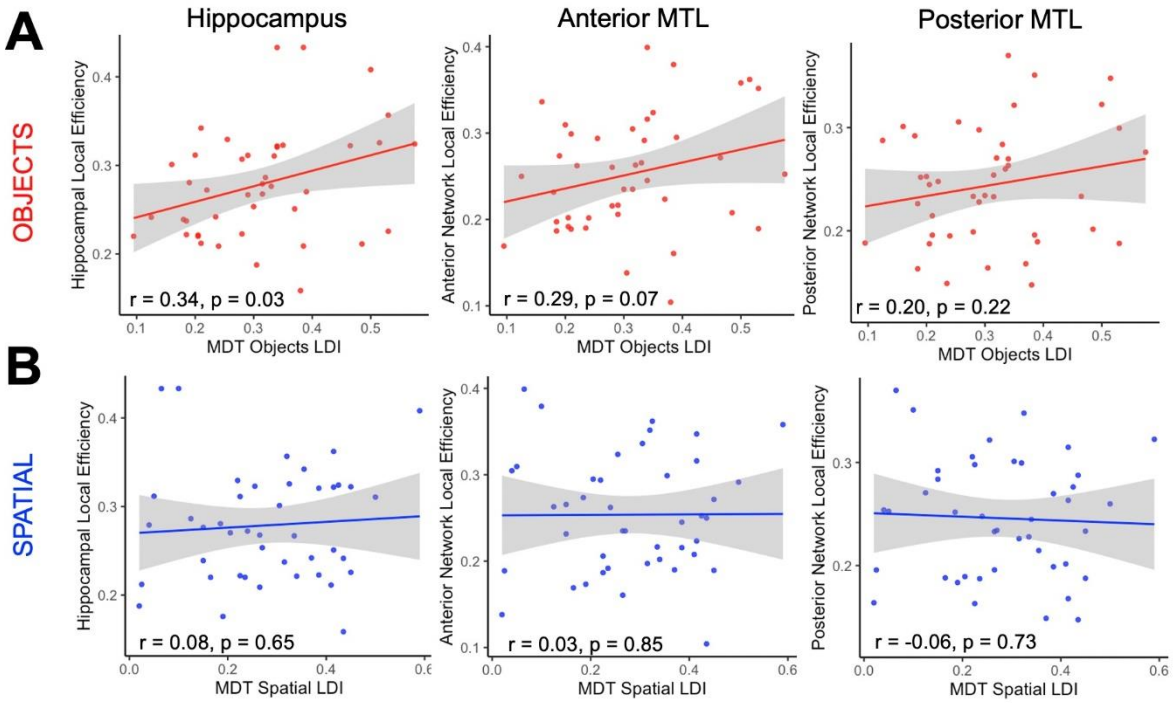
Topic: H.12. Aging and Development

Support: NIH R01 AG053555

Title: Medial temporal lobe network efficiency supports mnemonic discrimination of objects in older adults

Authors: *J. N. ADAMS, J. L. YAROS, S. KIM, L. MCMILLAN, M. A. YASSA; Neurobio. and Behavior, Univ. of California, Irvine, Irvine, CA

Abstract: The medial temporal lobe (MTL) is comprised of anterior and posterior functional networks that process object and spatial information, respectively, which converge in the hippocampus. We tested whether the local efficiency of these networks was related to mnemonic discrimination of object and spatial stimuli in cognitively normal older adults (OA). We hypothesized associations between anterior MTL local efficiency and object memory, posterior MTL local efficiency and spatial memory, and hippocampal local efficiency with both domains. Resting state fMRI was analyzed for 55 OA participants (72 ± 6 yrs; 67% F). Functional connectivity analyses were conducted in CONN across 22 unilateral MTL ROIs. Local efficiency was calculated with the Brain Connectivity Toolbox for each ROI and was averaged for the anterior MTL (perirhinal cortex, anterolateral entorhinal cortex, and amygdala), posterior MTL (parahippocampal cortex, posteromedial entorhinal cortex), and hippocampal (DG/CA3, CA1, subiculum) networks. OAs performed object and spatial versions of a mnemonic discrimination task (n=44), and performance was assessed with the lure discrimination index (LDI). Object mnemonic discrimination was more strongly related to local efficiency of the hippocampal ($r = 0.34$, $p = 0.03$) and anterior MTL ($r = 0.29$, $p = 0.07$) networks than the posterior MTL network ($r = 0.20$, $p = 0.22$; Figure 1A). Spatial mnemonic discrimination was not associated with the local efficiency of any network (ps>0.65; Figure 1B). Our results indicate local efficiency of MTL networks, specifically the hippocampal network, support mnemonic discrimination of object stimuli. As object processing is particularly vulnerable to aging, understanding the functional basis of this deficit is critical to preventing cognitive decline.



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Poster

P897. Learning and Memory in Aging: Non-Human Primates and Humans

Location: Virtual Only

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Program #/Poster #: P897.03

Topic: H.12. Aging and Development

Support: NIA P50 AG016573
NIA R01 AG053555

Title: Regional white matter hyperintensities are associated with reduced medial temporal lobe subregion volumes in older adults

Authors: *B. RIZVI[1], M. SATHISHKUMAR[1], F. MARQUEZ[1], S. J. GRANGER[1], L. MCMILLAN[1], A. M. BRICKMAN[2], N. J. TUSTISON[1,3], M. A. YASSA[1];
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Abstract: White matter hyperintensities (WMH) are visualized on a T2-weighted FLAIR image and are a radiological marker of small vessel cerebrovascular disease. WMH are associated with aging and clinical expression of Alzheimer's disease (AD), affecting the onset and progression of the disease. WMH are known to affect memory functioning in older adults, and one proposed mechanism by which they impact memory is via neurodegeneration of structures such as the entorhinal cortex and hippocampus. However, little is known about whether WMH affect specific medial temporal lobe (MTL) subregions that underlie episodic memory functioning, and how the spatial distribution of WMH plays a role in these relationships. We studied how total and lobar (frontal, temporal, parietal, occipital) WMH are associated with MTL subregion volumes. We derived total and lobar WMH volumes by processing T2-weighted FLAIR images using the MICCAI 2017 winning methodology, which was ported to the ANTsX ecosystem; the resulting labeled masks were segmented by a lobar atlas and further manually edited. Using T1 and T2-weighted images, the Automatic Segmentation of Hippocampal Subfields (ASHS) pipeline was used to derive labels and volumes of hippocampal and medial temporal lobe subregions. MTL subregion volumes were adjusted by total intracranial volume (TIV). 136 older individuals were included in these analyses (mean age (SD) = 76.32 (10.06) years; 90 (66%) women). After adjusting for age, sex/gender, and education, we found that total, frontal, parietal, and occipital WMH volumes were associated with reduced total Brodmann area (BA) 36 volume. Furthermore, occipital WMH was associated with lower total CA1, CA2, CA3, dentate gyrus (DG), entorhinal cortex (ERC), subiculum, and parahippocampal cortex TIV-adjusted volumes. These findings highlight the potential role of posterior WMH in promoting neurodegeneration of MTL subregions that have important roles in episodic memory.

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Poster

P897. Learning and Memory in Aging: Non-Human Primates and Humans

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Program #/Poster #: P897.04

Topic: H.12. Aging and Development

Support: NIH/NIA R01 AG053555 A1
NIH/NIA P50 AG016573

Title: Examining the diagnostic utility of the mnemonic discrimination task for classification of cognitive status and amyloid-b positivity

Authors: *S. KIM, M. G. CHAPPEL-FARLEY, D. KEATOR, J. JANECEK, L. MCMILLAN, B. A. MIRANDA, A. MIKHAIL, M. A. YASSA;
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Abstract: Deposition of beta-amyloid (Aβ) plaques is an early biomarker of Alzheimer's disease (AD), but current methods of measuring Aβ accumulation are invasive or expensive. Therefore, developing a non-invasive platform that can effectively predict Aβ burden is crucial, as this technology could help to identify individuals with increased risk for AD and provide a window for potential treatment or intervention. Mnemonic discrimination tasks (MDT) assess the ability to discriminate highly similar episodic memory representations (e.g., objects or spatial locations), and task performance is reliant on the DG/CA3 subregions of the hippocampus. Prior work has shown age-related impairment in object and spatial discrimination as well as hyperactivity within DG/CA3 subregions. The relationship between the MDT performance and Aβ burden, however, has not yet been directly tested. In this study, we investigated whether MDT performance can successfully classify cognitive status or Aβ load in older adults. A sample of 93 older adults (60-91 yrs old) with normal cognition and 10 older adults with a clinical diagnosis of Mild Cognitive Impairment (MCI) (62 -89 yrs old) underwent either cerebrospinal fluid (CSF) lumbar punctures or ^{18}F-florbetapir-PET imaging. Cognitively normal individuals were stratified into Aβ+ and Aβ- subgroups based on quantitative thresholds for Aβ positivity (SUVR >1.15, CSF $A\beta_{1\text{-}42/1\text{-}40} < 0.062$). Participants performed the object and spatial versions of the MDT. We used logistic regression and random forest algorithms, including MDT performance metrics and demographic factors (age, gender, education) as features to classify cognitive status (normal vs. MCI) and Aβ status (positive vs. negative). We evaluated the area under the receiver operating characteristic curve (AUC) using the classifiers in a 10-fold cross validation design. For classification of cognitive status, the best model performance was achieved by including all the features of object and spatial MDT metrics and demographics (AUC = 0.8). For classification of Aβ status in cognitively normal participants, object MDT metrics combined with demographics were the most effective features (AUC = 0.70). Our findings suggest that the object and spatial MDT may have clinical utility as a diagnostic tool to predict cognitive status and *in vivo* Aβ burden in preclinical older adults.

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Poster

P897. Learning and Memory in Aging: Non-Human Primates and Humans

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Program #/Poster #: P897.05

Topic: H.12. Aging and Development

Support: NIA R01AG053555

Title: Understanding the age-related decline in mnemonic lure discrimination performance using a hierarchical Bayesian diffusion model

Authors: *C. CHWIESKO[1], J. JANECEK[1], S. S. DOERING[1], J. VANDEKERCKHOVE[2], M. LEE[2], M. HOLLEARN[1], R. RATCLIFF[3], L. MCMILLAN[1], M. A. YASSA[1];
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Abstract: The number of older adults is rapidly rising in the world population. Episodic memory is impacted by age to varying degrees and is dramatically impaired in mild cognitive impairment and Alzheimer's disease. To better understand and distinguish healthy from pathological aging it is important to study normal age-related changes in episodic memory.The Mnemonic Discrimination Task (MDT), a modified object recognition memory task, which uses similar "lure" stimuli (perceptually similar but not identical images) has become a popular cognitive assay to study those changes. Several research groups have confirmed that older adults, in comparison to young adults, tend to identify similar lures more often incorrectly as old (i.e. overgeneralize). It is currently believed that this age-related decline in discrimination is due to a memory deficit, i.e. a reduction in pattern separation due to decreased hippocampal integrity. However, contributions of other cognitive processes to this age-related decline have not been thoroughly investigated. In the current study, we used a custom-designed hierarchical Bayesian diffusion model to investigate which cognitive processes contribute to the age-related decline in lure discrimination performance. The Ratcliff Diffusion model is a mathematical process model that allows us to decompose decision behavior in the MDT into the different underlying cognitive processes. For each of the model parameters (representing specific cognitive processes) we estimated the difference in the group means between older and younger adults across a series of hierarchical models. The best performing model was chosen based on the deviance information criterion and the group differences in the model parameters were evaluated using Bayesian statistics. Our results demonstrate strong evidence that age-related decline in discrimination performance is indeed based on reduced discrimination (i.e. memory) in older adults and not based on differences in other cognitive processes.

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Poster

P897. Learning and Memory in Aging: Non-Human Primates and Humans

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P897.06

Topic: H.12. Aging and Development

Support: NIA U01 AG051412

Title: Functional connectivity in the default mode network and medial temporal lobe network changes in the course of Alzheimer's disease in individuals with Down syndrome

Authors: *N. D. DIPROSPERO[1], M. SATHISHKUMAR[1], L. MCMILLAN[1], D. B. KEATOR[2], E. DORAN[3], C. HOM[2], D. NGUYEN[3], H. D. ROSAS[4], F. LAI[4], A. M. BRICKMAN[5], N. SCHUPF[5], W. SILVERMAN[3], I. T. LOTT[3], M. A. YASSA[1];
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Abstract: By age 40, virtually all people with Down syndrome (DS) have sufficient beta-amyloid and tau pathologies present to meet the criteria for pathological Alzheimer's disease (AD). Determining the timing and location of neurobiological changes caused by AD is critically important for effective intervention within this high-risk population and more broadly in the general population, but little is known about the brain changes associated with clinical onset of AD in DS. Previous studies in sporadic AD showed default mode network (DMN) hyperconnectivity in those with mild cognitive impairment (MCI) and hypoconnectivity in those with AD, likely driven by progression of amyloidosis. Additionally, evidence from neurotypical individuals suggests DMN functional connectivity with the medial temporal lobe (MTL) is vulnerable to AD-related impairment. The purpose of the present study is to determine how DMN and MTL functional connectivity differs in people with DS ages 40+ depending on dementia status.
One hundred seven individuals with DS (mean age 49.6±6.4, 38% women) from a subset of the Alzheimer's Biomarkers Consortium - Down Syndrome study underwent MRI, including a 1mm MPRAGE scan and a resting state fMRI scan (resolution varied between 2-3mm). Participants completed a battery of neuropsychological tests and were assigned a consensus diagnosis for the presence or absence of MCI or AD dementia. Seventy-one participants were classified as cognitively stable (CS) (mean age 48.7±6, 46% women), 23 participants had MCI-DS (mean age 51.1±5, 23% women), and 13 participants had AD dementia (mean age 56±6.4, 42% women). We hypothesized that participants with MCI-DS would have greater functional connectivity between the DMN and MTL compared to the CS and dementia groups. We also hypothesized that participants with DS who had dementia would have lower functional connectivity between the DMN and MTL compared to the CS and MCI-DS groups. We found that those with MCI-DS had greater functional connectivity between the posterior DMN and the posteromedial entorhinal cortex (pMEC) relative to the CS and dementia groups, in line with our hypothesis. However, those with dementia had lower functional connectivity within the MTL, specifically between the anterolateral entorhinal cortex (aLEC) and the pMEC, compared to the CS and MCI-DS groups. Changes in pMEC functional connectivity with the posterior default mode network and the medial temporal network track with the progression of clinical symptoms of AD-DS and may reflect synaptic changes stemming from AD pathology.

Disclosures: N.D. DiProspero: None. **M. Sathishkumar:** None. **L. McMillan:** None. **D.B. Keator:** None. **E. Doran:** None. **C. Hom:** None. **D. Nguyen:** None. **H.D. Rosas:** None. **F. Lai:** None. **A.M. Brickman:** None. **N. Schupf:** None. **W. Silverman:** None. **I.T. Lott:** None. **M.A. Yassa:** None.

Poster

P897. Learning and Memory in Aging: Non-Human Primates and Humans

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P897.07

Topic: H.12. Aging and Development

Support: NIA R01-AG049002

Title: Theta-specific noninvasive modulation of cerebellar contributions to episodic memory in healthy aging

Authors: *S. DAVE, S. L. WERT, E. CHUNG, S. VANHAERENTS, J. L. VOSS; Northwestern Univ., Chicago, IL

Abstract: The human cerebellum interacts with the large-scale brain network that supports episodic memory. A prevailing hypothesis suggests that these long-range interactions are guided by synchronous neural oscillations in function-specific frequency bands, e.g., at the theta (~3-8 Hz) frequency pattern dominant across hippocampal network regions during encoding and retrieval. Recent evidence in young adults supports this hypothesis (Dave et al., 2020), demonstrating that cerebellar contributions to episodic memory can be selectively enhanced by application of noninvasive brain stimulation (i.e., TMS) at a theta rhythm (theta burst) versus at a control beta-frequency rhythm. It is unclear whether similar rhythmic and location-specific factors can guide selective effects of cerebellar stimulation in older adults. Cognitively typical older adults show widespread and highly variable atrophy across the hippocampal-cortical memory network. In brain regions with relatively high levels of atrophy (e.g., the parietal cortex), selective effects of theta-patterned stimulation on episodic memory performance emerge in young adults but are not found in healthy older adults (Hermiller et al., 2021). As cerebellar volume is relatively better preserved in healthy aging, we investigated whether this site might serve as a location for influencing memory function. In 17 cognitively healthy older adults (9 women, ages 63-83), we found that cerebellar theta-burst TMS improved episodic memory encoding relative to both control-location theta-burst TMS of nearby occipital cortex ($p = 0.01$) and to control-rhythm beta TMS ($p = 0.05$). These findings suggest that the cerebellum be an ideal target for stimulation-based interventions for age-related memory impairment. Further, as is the case for younger adults, stimulation at theta rhythm is effective for episodic memory enhancement in older adults.

Disclosures: S. Dave: None. **S.L. Wert:** None. **E. Chung:** None. **S. VanHaerents:** None. **J.L. Voss:** None.

Poster

P897. Learning and Memory in Aging: Non-Human Primates and Humans

Location: Virtual Only

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Program #/Poster #: P897.08

Topic: H.12. Aging and Development

Support: NIGMS (P20GM103446) to CJC
NICHD (R21HD101964) to CJC
COBRE (5P20GM103653)

Title: Translating time across the lifespan in humans and chimpanzees

Authors: *J. SCHWARTZ[1], B. RIGBY DAMES[2], K. OFORI[1], C. BAUCUM[3], A. VAN DER KOUWE[4], E. TAKAHASHI[4], C. J. CHARVET[1];
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Abstract: How biological programs evolved to support human cognition is an enduring question of interest. Yet, we still lack appropriate procedures to align ages across species. We collected 228 time points from abrupt and gradual changes in behavior, neuroanatomy, and transcription in humans and chimpanzees to identify corresponding ages across the two species. We also collected time points (e.g., mortality curves) from diverse populations (e.g., hunter-gatherers) in males and females to account for individual variation in corresponding ages across humans and chimpanzees. Corresponding ages are similar in the two species early in development (shortly after birth) but gradually diverge with age in the two species. The lifespan is extended across diverse human populations relative to chimpanzees, and is concomitant with biological pathways (e.g., brain atrophy) clearly observed in aged humans but difficult to detect in chimpanzees. The inclusion of time points from diverse populations yields improved estimates in predicting ages in humans and chimpanzees, and shows that very old age as a distinctly human characteristic.

Disclosures: J. Schwartz: None. **B. Rigby Dames:** None. **K. Ofori:** None. **C. Baucum:** None. **A. van der Kouwe:** None. **E. Takahashi:** None. **C.J. Charvet:** None.

Poster

P945. New and/or Improved Methodology: Imaging, Biochemistry, Therapeutics

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P945.01

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

Title: Optimization of Protein Precipitation from Human Nails to Facilitate Early Detection of Mental Health Disorders

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Abstract: The National Alliance on Mental Illness (NAMI) reports that 1 in 5 adults will experience mental illness every year, and 1 in 20 adults will experience serious mental illness every year. 50% of lifetime disorders begin by the age of 14, and 75% of lifelong disorders begin by the age of 24. Early detection and intervention is key to improving patient outcomes, however research exploring potential biomarkers as early indicators is still limited. Thus far, biomarkers have been assessed almost exclusively in blood samples, which can be invasive to obtain and expensive to preserve and assess. The objectives of this project were twofold: optimization of protein precipitation in human nail samples, and identification and quantification of immune proteins present in human nails with potential to be biomarkers. We developed a one day extraction and precipitation protocol allowing for fast and efficient recovery of protein. Survey data and nail samples were collected from 68 individuals over a period of two months in the spring of 2021. Survey data (demographic information, GAD-7, PHQ-8, and TAPS-I) were used to categorize samples by screening tool scores. The optimized protein precipitation method was used to obtain protein pellets from participant nail samples. Protein concentration was ascertained using gel electrophoresis and Coomassie staining. Survey data responses were used to aggregate 1mg/mL stocks of extracted protein to detect immune cytokine presence via a Human XL Proteome Profiler Cytokine Array and were verified via Western blots. Future work aims to collect samples from psychiatric patients and utilize the same precipitation protocol as a comparison. The broader impact of this project is developing a resource to aid in working toward early detection and intervention for mental health disorders.

Disclosures: E. Moelker: None. **E. Ngun Cer Sung:** None. **N. Koorndyk:** None. **M. Stephan:** None. **P. Rivera:** None.

Poster

P945. New and/or Improved Methodology: Imaging, Biochemistry, Therapeutics

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Program #/Poster #: P945.02

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

Support: The Miami Project to Cure Paralysis
The Craig H. Neilsen Foundation
The Indiana Traumatic Spinal Cord and Brain Injury Research Fund from the Indiana State Department of Health

Title: An established workflow for the culturing of nerve-derived human Schwann cells: isolation, expansion, purification and quality control.

Authors: *P. V. MONJE[1], G. I. APARICIO[2,1], N. D. ANDERSEN[3], J. BRADBURY[1], K. WANCZYK[1], M. MURPHY[1];
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Abstract: We herein describe an optimized and scalable workflow to prepare and analyze cultures of human donor-derived Schwann cells (hSCs) from mature and developing nerve tissues. We recommend the following steps for optimal growth and purity of both the primary and established cell cultures: (1) pre-culturing perineurium-free nerve fibers in suspension and delaying tissue dissociation for at least one week to facilitate enzymatic cell release; (2) plating the initial cell suspensions in the form of individual droplets onto dishes double-coated with poly-L-lysine and laminin to increase the recovery of adherent cells; (3) introducing a step of purification via magnetic-activated cell sorting at passage-zero to rapidly and efficiently eliminate fibroblasts from the onset; and (4) culturing the tissues and the isolated cells in heregulin-supplemented medium to accelerate hSC mitogenesis before and after enzymatic dissociation. This protocol can render >100 million highly viable, proliferative hSCs depending on the size of the nerve biopsy and the level of amplification. The hSCs obtained in this manner are suitable for variety of downstream applications including cryopreservation and labeling using vital fluorophores, plasmids or lentiviral vectors. The cells are metabolically active, phenotypically stable, and capable to engulfing and degrading myelin even after undergoing 3 to 5 successive rounds of expansion. Nevertheless, heterogeneity and donor variability of individual cultures are expected. For this, we recommend a routine characterization of the populations to unequivocally identify non-glial cells, senescent cells, and cells at different stages of differentiation immediately after plating and at all levels of passage.

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Poster

P945. New and/or Improved Methodology: Imaging, Biochemistry, Therapeutics

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P945.03

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

Title: Identification of a novel blood-brain-barrier-penetrant AAV capsid in both rodents and primates

Authors: Y. YAO, J. WANG, Y. LIU, Y. QU, K. WANG, Y. ZHANG, Y. CHANG, Z. YANG, J. WAN, H. NAKASHIMA, S. E. LAWLER, C.-F. CHO, E. A. CHIOCCA, *F. BEI;
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Abstract: Recombinant adeno-associated viruses (rAAVs) have been increasingly explored as a vector for gene therapy in the central nervous system (CNS). Recently, we reported two new blood-brain-barrier (BBB)-penetrant AAV variants, namely AAV.CPP.16 and AAV.CPP.21, by engineering AAV9 capsid. Importantly, the superiority of AAV.CPP.16 over AAV9 translated from mice to non-human primates, showing 5-10 folds enhancement in CNS transduction with intravenous delivery. Here, we further report that both AAV.CPP.16 and AAV.CPP.21 showed enhanced transcytosis in human brain microvascular endothelial cells (hBMEC) in an *in vitro* BBB model. Increased viral bindings to hBMECs by both variants were observed. In addition, increased transduction of and viral binding to primary human astrocytes were also observed for both AAV variants. These results point to potential factor(s) in cytoplasmic membranes of endothelial cells and CNS cells that could account for enhanced CNS transduction by the variants. Furthermore, to demonstrate proof-of-concept for application in CNS gene therapy, we utilized AAV.CPP.16 to develop a systemic, PD-L1-antibody-based gene therapy that had a significant pro-survival effect in a mouse model of glioblastoma. In comparison with AAV9, AAV.CPP.16 improved systemic delivery of the PD-L1 antibody to the tumor and thus increased infiltration of $CD3^+$ lymphocytes and $CD8^+$ cytotoxic T cells in the tumor microenvironment. In summary, our follow-up studies provide more mechanistic insight into the newly engineered, BBB-penetrant AAV variants, and demonstrate their prospect for translational applications in CNS gene therapy.

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Poster

P945. New and/or Improved Methodology: Imaging, Biochemistry, Therapeutics

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Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P945.04

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

Support: NIMH R01MH122742

Title: Development of conjugate vaccines providing differential selectivity profiles against ketamine and 6-hydroxynorketamine

Authors: Z. ZHENG, **J. L. KYZER**, A. WOROB, *C. J. WENTHUR;
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Abstract: While the anesthetic drug ketamine has dissociative and hypnotic properties that drive its misuse and abuse, it also has rapidly acting antidepressant properties that have supported its use as a breakthrough therapy in treatment resistant depression. Given this mixed risk/benefit profile, there has been significant interest in identification of distinct mechanisms that support ketamine's antidepressant activity without inducing rewarding or dissociative effects. Notably, ketamine administration *in vivo* results in coincident exposure to multiple active metabolites, including norketamine and multiple 6-hydroxynorketamine diastereomers. Therefore, to enable assessment of the functional contributions from each of these molecular species, we aimed to generate highly selective anti-drug immunoglobulins that could prevent each of these small molecule targets from crossing the blood-brain barrier to elicit their psychoactive effects. We first used synthetic organic chemical approaches to generate three novel bioconjugate haptens, KET-N-COOH, NK-N-COOH, and 6-HNK-N-COOH. Vaccination of mice with these bioconjugates, co-formulated with alum and CpG ODN 1826, was then undertaken using a 28-day, three injection protocol. Serum antibody titers were assessed using an enzyme-linked immunosorbent assay (ELISA) against KET-BSA, NK-BSA, and HNK-BSA coated plates. Midpoint titers from CRM-KET and CRM-NK were highest against KET-BSA, while those from CRM-HNK were highest against HNK-BSA. The resulting IgG antibodies were isolated from serum and their affinity was assessed using competitive surface plasmon resonance (SPR) measurements against ketamine, norketamine, or 6-hydroxynorketamine. Antibodies from CRM-KET sera demonstrated an affinity ratio of approximately 1:90:1 for ketamine, norketamine, and 6-hydroxynorketamine; this ratio was 1:16:1 for CRM-NK antibodies; and 33:105:1 for CRM-HNK antibodies. Mice were challenged with 0 - 50 mg/kg ketamine, and ketamine's effects in the open field test, tail flick test, and hotplate test were found to be blunted by vaccination with CRM-KET. Overall, these novel vaccines have demonstrated the potential to enable mechanistic assessment of metabolite-driven polypharmacologic contributions to ketamine's rewarding and antidepressant effects, and may potentially be useful for therapeutic reversal of ketamine overdose.

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Poster

P945. New and/or Improved Methodology: Imaging, Biochemistry, Therapeutics

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P945.05

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

Title: Understanding Kinases Important in GBM Survival

Authors: *K. CHUGH[1], Z. SHENG[2];
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Abstract: BACKGROUND: Glioblastoma (GBM) is the most common and deadly brain tumor in adults with a 5 year survival rate of 5%. Even with aggressive treatment, the prognosis of GBM is poor. Current standard of care for chemotherapy is with TMZ, a methylating agent. However, at least 50% of patients treated with TMZ do not respond to it. It is therefore necessary to identify other possible therapies for GBM.
OBJECTIVE: The primary objective of this study is to identify existing chemotherapies that may have a role in regulating the expression of genes that are necessary for GBM survival (known as survival kinase genes). We hypothesize that current existing chemotherapies, particularly those that target EGFR, will be negatively correlated with survival kinase gene expression.
METHODS: Survival kinase gene expression, chemotherapeutic agents and sensitivity of GBM cells (IC50) from 27 cell lines were obtained from data base The Cancer Dependency Map. The 15 survival kinase genes for GBM were established from previous studies.
RESULTS: Expression for 14 of the 15 survival kinase genes studied were significantly positively or negatively correlated with an existing therapeutic agent. Negative correlation indicated that cells with a high level of that survival kinase are sensitive to the given chemotherapeutic agent. Positive correlation indicated that GBM cells with a high expression of a particular survival kinase were not sensitive, and therefore may not be a good target for treatment. It was identified that cells with a high level of expression of survival kinase gene ROR2 had the most chemotherapeutic agents negatively regulating gene expression, while MAP4K3 had 5 therapies that were positively correlated, indicating that this site may not be sensitive to chemotherapeutic treatment. Furthermore, drugs that targeted c-MET, XIAP, CDK41and EGFR are consistently negatively correlated with expression of their respective survival kinase gene. This research is exploratory.
FUTURE DIRECTIONS: Given these significant correlations, drugs that target c-MET, XIAP, CDK4 and EGFR may be useful areas to research further and target as therapies for GBM. Drugs that targeted TOP1 sites (Irinotecan and topotecan) were consistently positively correlated with their survival gene expression, indicating that their respective kinases may be a poorer prognostic marker and target for treatment. Given that these are correlations, next steps include an *in vitro* study to understand the viability of cells with both high and low levels of the given survival gene in response to the chemotherapeutic compounds, to ensure that high expression of that given gene indicates a sensitivity to the compound.

Disclosures: K. Chugh: None. **Z. Sheng:** None.

Poster

P945. New and/or Improved Methodology: Imaging, Biochemistry, Therapeutics

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:00 PM - 3:00 PM

Program #/Poster #: P945.06

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

Title: Multiplexed multispectral imaging to visualize cancer cell subtypes and evaluate the immune landscape in glioblastoma

Authors: *A. E. HAGGERTY[1], N. MAMMADOVA[1], C. E. BROWN[2], M. E. BARISH[3]; [1]Akoya Biosci., Marlborough, MA; [2]City of Hope, Duarte, CA; [3]Neurosciences, Beckman Res. Inst. City of Hope, Duarte, CA

Abstract: Abstract: Glioblastoma (GBM) is an aggressive and almost universally fatal brain cancer. Glioblastoma tumor masses are highly vascularized and infiltrated with cells mediating both innate and adaptive immunity. The presence and organization of various immune cells within the tumor microenvironment varies between patients and within individual tumors. This variability in the immune landscape influences responses to different treatment modalities, including immunotherapies. Understanding reciprocal immune-tumor cell interactions will enhance design and implementation of novel therapies. Currently, review of GBM patient tissues by neuropathologists is done visually using single or low-plex biomarker panels whose complexity is constrained by limitations of traditional immunohistochemistry, including the lack of well characterized primary antibody clones, limited antibody selection due to species-dependent workflows, and inaccuracies such as false signal intensities introduced by autofluorescence (AF) and spectral overlap from traditional immunofluorescence imaging methods. Multiplex immunofluorescence (mIF) and multispectral imaging (MSI) allows for fast, accurate imaging on a single tissue section of up to eight fluorophores (using Phenoptics technology), cell type-specific AF spectra, and DAPI/Hoechst. Of note, purported cancer stem cells have been shown to have unique AF signatures that can be isolated utilizing MSI technology in the context of mIF and nuclear markers. Utilizing MSI technology and spectral unmixing leads to improved signal-to-noise ratios and accuracy of cellular phenotyping, ultimately culminating in greater data confidence and reproducibility. Here, we have used pre-validated mIF kits that use Opal fluorophores together with the Vectra Polaris Automated Quantitative MSI system on human formalin-fixed, paraffin embedded GBM tissue samples to further characterize immune-tumor microenvironments, with consideration for spatial distributions of interacting cells. Pre-optimized immunofluorescent kits targeting immune cell subsets, checkpoint inhibitors, and other biomarkers have been shown to provide clinically relevant information in other cancer types (such as lung cancer and melanoma). Our study indicates that widespread use of these reagents would better characterize the complex immune landscape of tumors and enhance design and implementation of therapeutics for GBM.

Disclosures: A.E. Haggerty: A. Employment/Salary (full or part-time):; Akoya Biosciences. **N. Mammadova:** A. Employment/Salary (full or part-time):; Akoya Biosciences. **C.E. Brown:** None. **M.E. Barish:** None.

Poster

P945. New and/or Improved Methodology: Imaging, Biochemistry, Therapeutics

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

Support: R01GM128997
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R01MH101214
R01MH12478

Title: Psychedelic-inspired drug discovery using an engineered biosensor

Authors: *C. DONG[1], C. LY[2], L. E. DUNLAP[2], M. V. VARGAS[2], J. SUN[2], I.-W. HWANG[3], A. AZINFAR[2], W. OH[3], W. C. WETSEL[4], D. E. OLSON[2], L. TIAN[1];
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Abstract: Ligands can induce G protein-coupled receptors (GPCRs) to adopt a myriad of conformations, many of which play critical roles in determining the activation of specific signaling cascades associated with distinct functional and behavioral consequences. For example, the 5-hydroxytryptamine 2A receptor (5-HT2AR) is the target of classic hallucinogens, atypical antipsychotics, and psychoplastogens. However, currently available methods are inadequate for directly assessing 5-HT2AR conformation both in vitro and in vivo. Here, we developed psychLight, a genetically encoded fluorescent sensor based on the 5-HT2AR structure. PsychLight detects behaviorally relevant serotonin release and correctly predicts the hallucinogenic behavioral effects of structurally similar 5-HT2AR ligands. We further used psychLight to identify a non-hallucinogenic psychedelic analog, which produced rapid-onset and long-lasting antidepressant-like effects after a single administration. The advent of psychLight will enable in vivo detection of serotonin dynamics, early identification of designer drugs of abuse, and the development of 5-HT2AR-dependent non-hallucinogenic therapeutics.

Disclosures: C. Dong: None. **C. Ly:** None. **L.E. Dunlap:** None. **M.V. Vargas:** None. **J. Sun:** None. **I. Hwang:** None. **A. Azinfar:** None. **W. Oh:** None. **W.C. Wetsel:** None. **D.E. Olson:** None. **L. Tian:** None.

Poster

P945. New and/or Improved Methodology: Imaging, Biochemistry, Therapeutics

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Title: Two-photon imaging of rapid voltage dynamics in mice and flies with JEDI indicators

Authors: *X. LU[1], Z. LIU[2], Y. GOU[3], M. LAND[4], S. LAI[4], K. L. COLBERT[4], J. LEE[1], T. ASSEFA[5], M. M. KORYMPIDOU[6], A. VLASITS[6], E. FROUDARAKIS[3], N. ZHOU[3], S. PATEL[3], M. M.-H. PANG[7], S. SU[8], V. VILLETTE[9], J. BRADLEY[9], P. BIZOUARD[9], T. CLANDININ[10], K. FRANKE[11], J. REIMER[3], A. S. TOLIAS[3], S. DIEUDONNE[9], F. ST-PIERRE[3];
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Abstract: A longstanding goal in neuroscience is to understand how spatiotemporal patterns of neuronal electrical activity underlie brain function, from sensory representations to decision making. An emerging technology for noninvasive monitoring membrane electrical dynamics is voltage imaging using Genetically Encoded Voltage Indicators (GEVIs) — light-emitting protein indicators whose brightness reports membrane voltage. GEVIs are promising tools for monitoring voltage dynamics at high spatiotemporal resolution in genetically defined cell types in vivo. While significant screening efforts had been made to improve sensors' performance, existing pipelines are usually restricted to evolve one or a few properties of GEVIs, leading to biased champions that do not enable robust single-trial two-photon imaging of voltage dynamics in behaving rodents. Therefore, GEVIs have yet to be broadly adopted. To fill in the gap between the demand for robust neuron imaging and the imperfect performance of existing GEVIs, we developed a pipeline for high-throughput screening of GEVI libraries, followed by detailed characterization of the most promising candidates. Here, we will present our efforts on developing the screening platform to evolve GEVIs in all performance metrics and semi-rational engineering of GEVIs guided by structure and evolutionary studies, using indicators of the

Accelerated Sensor of Action Potential (ASAP) family as a starting point. The best indicator identified during our screening, which we named Jellyfish-derived Electricity-reporting Voltage Indicators (JEDIs), were used report light-evoked graded potentials with subcellular resolution in mice retinal explants and living flies. JEDI also enabled single-trial recording of spiking activity in awake behaving mice with both resonant scanning and ULoVE random-access microscopy. These results demonstrate the utility of JEDI across model systems and imaging modalities, paving the way for its broader deployment by the neuroscience community.

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Poster

P945. New and/or Improved Methodology: Imaging, Biochemistry, Therapeutics

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Klingenstein-Simons Fellowship Award in Neuroscience

Title: JEDI2: A second-generation indicator for two-photon imaging of voltage dynamics

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Abstract: Genetically Encoded Voltage Indicators (GEVIs) are emerging tools for non-invasive monitoring of voltage dynamics in genetically defined cell types. GEVIs promise to enable a wide range of applications from detecting changes in the membrane potential of subcellular compartments to monitoring large scale neuronal activity *in vivo*. However, current sensors suffer from low sensitivity to changes in membrane potential and are relatively dim, limiting our

ability to image smaller transients and voltage dynamics in deeper layers of the cortex. Finally, poor photostability of previously reported GEVIs means these sensors can only be used to image continuously for a few minutes and thus cannot be used to measure neuronal activity during many standard behavioral paradigms. Here, we report novel GEVIs optimized for long-term *in vivo* imaging under two-photon illumination, a method of choice for deep-tissue imaging. Our starting point was JEDI (see separate poster from Xiaoyu Lu et al.), a sensor based on the ASAP family of sensors in which a voltage-sensitive domain (VSD) is coupled to an extracellular circularly permuted GFP (cpGFP). Using a high-throughput screening platform, we screen variants with mutations in the cpGFP and the VSD, including junction points between these two domains. Using this platform, we identified a next-generation GEVI named Jellyfish-derived Electricity-reporting Designer Indicators 2 (JEDI2). JEDI2 shows significant improvements in brightness and response amplitude as compared with our first-generation sensor, JEDI. We anticipate this sensor will facilitate more robust high-throughput and real-time neural recording over longer time-spans, thus advancing our understanding of neuronal processing in health and disease.

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Poster

P945. New and/or Improved Methodology: Imaging, Biochemistry, Therapeutics

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Program #/Poster #: P945.10

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

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Title: Ultra-photostable fluorescent proteins engineered using versatile phenotype-activated cell sorting.

Authors: *J. LEE[1], J. AHRENS[1], Z. LIU[1], M. LEE[1], P. SUZUKI[2], S. LAI[3], X. LU[1], A. MCDONALD[3], S. GUAN[5], F. ST-PIERRE[4];
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Abstract: Understanding brain development and neuronal disease progression requires imaging over hours, weeks, or even months. Fluorescent proteins (FPs) are commonly used to study these processes by reporting changes in cellular morphology, gene expression, or protein localization. However, the low brightness and photostability of many FPs limit the duration or temporal resolution of longitudinal studies. Therefore, it is imperative to engineer brighter and more photostable FPs. The traditional approach for FP engineering is to use directed evolution in *Escherichia coli*. This process is tedious and low throughput. Furthermore, the performance improvements of FPs developed in bacteria and characterized *in vitro* may not necessarily apply when expressed in neurons. To address these issues, we developed SPOTlight, an imaging-based single-cell platform for screening both brightness and photostability in eukaryotic cells. SPOTlight can screen millions of cells immobilized in a culture dish and retrieve the desirable variants by *in situ* optical tagging. We have fully automated the screening process using only commercially available software and hardware, allowing easy implementation and dissemination of the platform. Furthermore, the platform supports the versatile nature of microscope-based screening as it can work with different cell types: bacteria, yeast, mammalian cells, and organoids. We successfully utilized the platform to screen millions of yellow FP (YFP) variants for improved brightness and photostability. Using SPOTlight, we engineered a new YFP variant, mGold2, that is around ten times more photostable than commonly used YFPs such as mVenus. We anticipate broad adaption of mGold2 as a visualization probe for longitudinal studies. We also believe that SPOTlight will be rapidly adapted for a wide range of applications for retrieving rare cells based on spatial or temporal phenotypes.

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Poster

P981. Electrical Methods to Modulate Neural Activity V

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Program #/Poster #: P981.01

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH CTSA UL1TR002553
USAMRAA-CDMRP EP200077
NSF CBET-1752274

Title: High-density, Micro-contact Stereo-EEG Array for Submillimeter Monitoring of Epileptic Activity

Authors: *I. RACHINSKIY[1], C. CHIANG[1], C. WANG[1], M. TRUMPIS[1], S. DURAIVEL[1], K. BARTH[1], W. LU[1], S. R. SINHA[2], D. G. SOUTHWELL[3], G. B. COGAN[4], J. VIVENTI[1];

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Abstract: Epilepsy surgery can be an effective treatment against refractory epilepsy, which occurs in 30% of epilepsy cases is resistant to pharmaceutical treatments. However, surgical resection halts seizures only in about 60% of cases. Due to the low level of successful outcomes and severity of the procedure, epilepsy surgery remains underused. Surgical outcomes are governed by the ability to locate and completely remove the seizure onset zone (SOZ). Stereo-EEG (SEEG) is a common pre-surgical brain mapping procedure used to locate the SOZ and eloquent cortex by temporarily implanting depth electrodes and recording ictal and inter-ictal activity. Pre-surgical mapping governs resection location and extent, but is often imprecise due to large electrode size and sparse electrode density. This can lead to incomplete removal of epileptic tissue and therefore continuation of seizures. To enhance pre-surgical mapping techniques and improve surgical outcomes, we have designed a hybrid SEEG electrode array with both cylindrical macro (2.4x1.3mm size, 5mm pitch) and round micro (200µm size, 500µm pitch) contacts along the shank. The electrode arrays were fabricated in the Shared Materials Instrumentation Facility using a standard lithographic process on thin film liquid crystal polymer substrate and rolled into a cylindrical shape that mimics the dimensions and form-factors of standard SEEG electrodes. Macro contacts mimic current SEEG devices while micro contacts provide higher density sampling and smaller contact size, enabling collection of rich neural signals with higher spatial resolution and improved sensitivity to high frequency activity, including high-frequency oscillations and micro-seizures. Additionally, having electrodes in a 3-dimensional configuration around the shank could help in localizing signal sources, as compared to standard cylindrical electrodes. Electrode impedances at 1kHz were 1kΩ and 20kΩ for the macro and micro electrodes respectively. We tested the rolled devices in a rat model, recording evoked activity over the auditory cortex while presenting sound stimuli. The electrodes showed ability to decode stimulus tones on the auditory cortex at an accuracy of 69% (8% chance) in the high-gamma (HG) power envelope (70-150Hz). Meanwhile, standard SEEG recordings in patients had far lower signal to noise ratio in the higher frequency bands than micro-electrodes, 2dB and 12dB respectively. This novel electrode design could improve SOZ mapping and surgical outcomes. *Parts of the technology described are patent pending: "Electroencephalography (EEG) Electrode Arrays and Related methods of Use" U.S. Patent App. #PCT/US2020/051400.*

Disclosures: I. Rachinskiy: None. **C. Chiang:** None. **C. Wang:** None. **M. Trumpis:** None. **S. Duraivel:** None. **K. Barth:** None. **W. Lu:** None. **S.R. Sinha:** None. **D.G. Southwell:** None. **G.B. Cogan:** None. **J. Viventi:** None.

Poster

P981. Electrical Methods to Modulate Neural Activity V

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Program #/Poster #: P981.02

Topic: I.08. Methods to Modulate Neural Activity

Support: NSF GRFP DGE-1644868
NIH 1U01NS123668
NSF CBET-1752274

Title: Design and simulation of an actively multiplexed 2500 channel micro-electrocorticography (μECoG) array

Authors: *G. SHULL[1], T. JOCHUM[1], C. CHIANG[1], J. C. MORIZIO[2], J. VIVENTI[1];
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Abstract: High-density, micro-electrocorticography (μECoG) provides the ability to resolve fine features of local field potentials (LFPs) from the surface of the brain. These signals have been used to decode motor intention in brain computer interfaces (BCIs), and to control an exoskeleton in a patient with paraplegia. Additionally, μECoG can record multi-unit activity from the cortex if the electrodes are sufficiently small (<~1000 μm^2). However, creating small dense arrays limits the number of channels and recording area due to constraints imposed by connectors and individually wired electrodes and thus limits the performance of μECoG-based BCIs.Active μECoG arrays have significant potential to address this limitation using transistor-based time division multiplexing (TDM). TDM is implemented by adding a switch transistor to each recording site to allow multiple channels to share the same output, effectively increasing the number of recording channels without increasing the number of output wires. This enables high-density recordings without compromising the recording area. A significant limitation of TDM based active μECoG is the additional noise (~50 μV rms in 0.1 - 10KHz) caused by 1) TDM-based aliased thermal noise, 2) fabrication-based defects due to university clean room processing, and 3) transistor-based noise. The present work sought to design a 2500 channel μEcoG array with 50 μm pitch electrodes to address these challenges by adding filters, an amplifier, and a buffer before the multiplexing switch transistor at each electrode recording site. To accomplish this, we leveraged recent developments in commercial complementary-metal-oxide-semiconductor (CMOS) foundry fabrication combined with post-fabrication processing to create flexible electronics. This lowered noise from fabrication defects, while our design of a novel amplifier lowered noise due to aliasing and minimized transistor noise. Using simulations, we iterated through various amplifier architectures and achieved input-referred noise values of 10 μV rms in in the band 0.3 - 200 Hz, 11 μV rms in 300 - 3000 Hz. The amplifier gain was 40 dB, bandwidth 0.3 - 10 KHz, and 0.8 μW/channel power consumption. We will next fabricate the design and perform in vitro and in vivo testing. This electrode array will expand the capabilities of μECoG by enabling recording from the cortex with low noise, high resolution, and broad coverage.

Disclosures: G. Shull: None. **C. Chiang:** None. **J.C. Morizio:** None. **J. Viventi:** None.

Poster

P981. Electrical Methods to Modulate Neural Activity V

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Program #/Poster #: P981.03

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH CTSA UL1TR002553
NSF CBET-1752274
NIH 1UG3NS120172

Title: Micro-electrocorticography arrays capture micro-scale spatiotemporal dynamics of high-frequency oscillations in intraoperative recordings of human epilepsy patients

Authors: *K. J. BARTH[1], C.-H. CHIANG[1], S. RAHIMPOUR[4], J. SUN[8], M. TRUMPIS[1], C. WANG[1], S. DURAIVEL[1], A. DUBEY[8], K. WINGEL[8], I. RACHINSKIY[1], W. DOYLE[9], D. G. SOUTHWELL[4,5], M. HAGLUND[4], A. H. FRIEDMAN[4], S. P. LAD[4], F. SOLZBACHER[10,11,12], S. DEVORE[13], O. DEVINSKY[14,9,13], S. R. SINHA[6,7], G. B. COGAN[4,7,2,3], D. FRIEDMAN[13], B. PESARAN[8,13], J. BLANCO[15], J. VIVENTI[1,4,5,7];
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Abstract: Epilepsy affects over 65 million people, but anti-seizure medications alone fail to control seizures in ~30% of cases. Two alternative treatment options are surgical removal of seizure-generating brain tissue or implantation of a responsive neurostimulator. The success of either treatment depends on accurate identification of the seizure onset zone (SOZ), or the area of brain from which seizures are generated. However, precise SOZ localization is limited by a mismatch between the fine micro-scale electrophysiology of epilepsy and the coarse spatial scale of clinical recording arrays. Specifically, long-standing research from penetrating micro-electrode arrays (MEAs) has demonstrated that epileptic signals initiate in confined brain areas of less than $1mm^2$ (Schevon, et al. 2008). Past research has also demonstrated a relationship between the SOZ and interictal events recorded in the local field potential (LFP), such as high-frequency oscillations (HFOs, 80-500 Hz) (Staba, et al. 2014). However, the micro-scale spatiotemporal dynamics of HFOs in the epileptic brain are not well understood. We have sought to determine the spatial scale of HFOs in human epilepsy and evaluate how micro-scale recordings of epileptic tissue may provide a more robust picture of HFO activity. We used novel micro-electrocorticography (μECoG) arrays made of a flexible liquid crystal polymer thin film

(LCP-TF) substrate to record intraoperatively from human subjects with epilepsy undergoing resective surgery (Chiang, et al. 2021). Our µECoG arrays have between 244 - 512 recording sites with coverage between 16 mm^2 - 1560 mm^2, thus offering both high resolution recordings while maintaining spatial coverage comparable to standard clinical ECoG grids. We successfully identified HFOs in our µECoG recordings using an automated detection algorithm along with automated and visual artifact rejection. We found that the rate of HFOs varies between recording sites, with specific regions of elevated HFO activity. Through analysis of signal covariance between HFO events and surrounding activity, we found a majority of HFOs occurred on single channels. For those events occurring on multiple channels, the average spatial extent of the HFOs fell between 0.8 - 6.8 mm, which was variable between subjects. Our results indicate that HFOs occur in micro-domains and that micro-scale recordings of HFOs may offer a more accurate tool for SOZ localization and improved epilepsy treatment. Parts of the technology described here are patent pending under "Electroencephalography (EEG) Electrode Arrays and Related Methods of Use" U.S. Patent Application # PCT/US2020/051400.

Disclosures: K.J. Barth: None. **C. Chiang:** None. **S. Rahimpour:** None. **J. Sun:** None. **M. Trumpis:** None. **C. Wang:** None. **S. Duraivel:** None. **A. Dubey:** None. **K. Wingel:** None. **I. Rachinskiy:** None. **W. Doyle:** None. **D.G. Southwell:** None. **M. Haglund:** None. **A.H. Friedman:** None. **S.P. Lad:** None. **F. Solzbacher:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Blackrock Microsystems. **S. Devore:** None. **O. Devinsky:** None. **S.R. Sinha:** None. **G.B. Cogan:** None. **D. Friedman:** None. **B. Pesaran:** None. **J. Blanco:** None. **J. Viventi:** None.

Poster

P981. Electrical Methods to Modulate Neural Activity V

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Program #/Poster #: P981.04

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH CTSA UL1TR002553
NSF CBET-1752274
NIH 1UG3NS120172

Title: Speech decoding from human sensorimotor cortex using high-resolution µECoG

Authors: *S. DURAIVEL[1], S. RAHIMPOUR[3], C.-H. CHIANG[1], M. TRUMPIS[1], C. WANG[1], K. BARTH[1], D. SOUTHWELL[4], S. LAD[5], A. FRIEDMAN[3], S. SINHA[6], J. VIVENTI[2], G. COGAN[7];
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Abstract: Previous work on speech decoding using cortical signals demonstrated that the human sensorimotor cortex encodes kinematic properties of speech motor articulation (Chartier *et al.*, 2018). Effective decoding of these features combine to form elemental phonemic sounds which can be used to develop speech prostheses using electrocorticography (ECoG). Standard clinical ECoG, however, is limited by low spatial resolution (4 - 10 mm) and this limitation is evident from studies that estimate distinctive neural signals at resolutions below 4 mm (Muller *et al*., 2016). This suggests a need for higher density neural recordings to enable more robust speech decoding for neural prostheses. In this study, we demonstrated that high-density micro-ECoG (µECoG) electrodes can decode spoken speech. We recorded cortical signals using a 128 channel array (11 x 21 mm coverage, 1.33 mm electrode spacing) placed on the sensorimotor cortex of two Parkinsonian patients during awake DBS implantation. The subjects repeated a set of auditorily presented single-and bi-syllabic non-words. Our recordings revealed a significant increase in high gamma power (HG: 70-150 Hz) compared to baseline in at least 110 out of 128 channels for both subjects. We identified that HG signal to noise ratio is significantly higher in µECoG (5 dB) electrodes compared to standard ECoG and SEEG (2.5 dB). To decode individual speech sounds, we constructed a linear model using HG power for each phoneme position in the non-word. Results demonstrate above-chance decoding performance in predicting phonemes across syllabic positions for both subjects (S1: 48.7 ± 9%, S2: 55.98 ± 6.2%; chance: 11.11%), and significantly higher decoding compared to standard ECoG (29.21 ± 4.3%). Further, we observed that the model's decoding error is predominated by confusing phonemes that use similar articulators. These neural activation patterns also demonstrate spatial clustering patterns for articulators, revealing high-resolution phonetic feature cortical maps. In the time domain, we created a moving-window decoding model which shows that the peak decoding ability was sequentially dependent on syllabic-positional encoding of phonemes. Subsequently, using these sequential activations, we created a recurrent-neural-net phoneme sequence generator that can simultaneously generate phonemes with an average decoding accuracy of 46.3% and 50% (chance = 11.11%) for the first and second subjects respectively. Overall, we show the importance of high-resolution cortical sampling using µECoG electrodes for effective speech prosthetic technologies.

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Poster

P981. Electrical Methods to Modulate Neural Activity V

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Program #/Poster #: P981.05

Topic: I.08. Methods to Modulate Neural Activity

Title: High-performance, Conformable, Stencil Fabricated Graphene µ-ECoG Arrays

Authors: *J. HU[1], R. HOSSAIN[1], Z. NAVABI[1], A. TILLERY[2], S. KODANDARAMAIAH[1]; [1]Mechanical Engin., The Univ. of Minnesota, Twin Cities, Minneapolis, MN; [2]Bioengineering, Univ. of Maryland, Col. Park, College Park, MD

Abstract: Minimally invasive, flexible, electrocorticography (ECoG) arrays have the ability to monitor surface field potentials across large areas of the cortical surface and have revealed key insights on how neuronal activity distributed across multiple brain regions mediating behavior. However, ECoG devices are commonly fabricated using microfabrication techniques in a cleanroom environment, which greatly hinders the accessibility and cost-effectiveness of the device to the neuroscience labs lacking the facilities and skills. We have developed a fully desktop-fabricated stencil-based ECoG sensor. A customize synthesized Cyrene-graphene ink was deposited on a flexible 50 µm thick PI film using a laser-cut stencil to pattern the electrodes, which was subsequently encapsulated by spin-coated PDMS for insulation and protection. Using this procedure, we achieved 16-channel flexible ECoG sensors with inter-electrode pitch of 450µm and individual electrode/tissue interface area of diameter 200 µm. We performed benchtop tests to evaluate the ECoG sensor's electrical performance. A corrosion test on a 16-channel ECoG sensor was conducted in 65°C saline for 15 days where the impedances of all the 16 channels reduced at least by an order of magnitude from its initial values on day 1. Electrode impedances at 1000 Hz were measured in saline prior to implantation indicated average impedances of 64.05±58.5 KΩ (n=4 devices, 48 working channels) with a maximum impedance of 161.61 KΩ. The devices were chronically implanted for up to 72 days on 4 wild-type mice C57/BL6 mice with all devices having 16 functioning channels measuring average impedances of 73.50±49.113 KΩ post-implantation. Presentation of whisker stimulus in awake mice resulted in robust activation of electrodes in the contralateral hemisphere. The electrodes had an average signal-to-noise-ratio (SNR) of 3.08±0.80 when measuring sensory stimulus-evoked activity. The simple desktop fabrication methodology can be easily deployed in everyday neuroscience laboratories to create and use these high-performance flexible graphene ECoG electrode arrays.

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Poster

P981. Electrical Methods to Modulate Neural Activity V

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Topic: I.08. Methods to Modulate Neural Activity

Support: Weill Neurohub
University of Washington Royalty Research Fund
NSF Grant EEC-1028725
Washington Research Foundation
NSF Graduate Research Fellowship Program (GRFP)

Title: The mechanisms of plasticity following ischemic stroke and cortical stimulation in non-human primates

Authors: *J. ZHOU[1], K. KHATEEB[1], Z. IP[1], A. YAZDAN-SHAHMORAD[2];
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Abstract: Brain plasticity has been widely observed during behavioral tasks and functional recovery after damage. As a result, brain stimulation protocols can be designed to take advantage of this natural plasticity and serve as a potential therapy for various disorders. In particular, ischemic stroke is a major cause of brain injury that results in lifelong disability. Although past works in rodents have demonstrated protective effects and neuroplasticity induced by stimulation following stroke, few of these results have been replicated in humans due to the huge anatomical differences between rodent and human brains and a limited understanding of the underlying plasticity mechanisms. Therefore, we combined electrophysiology and immunohistochemistry to study the mechanisms of neuroplasticity following cortical ischemic stroke and electrical stimulation in non-human primates (NHPs). To produce controlled focal lesions, we used the photothrombotic method on the cortical surface to cause targeted vasculature damage. In two adult Rhesus Macaques, we generated an ipsilateral lesion in the sensorimotor cortex while collecting electrocorticography (ECoG) signals bilaterally. In another two animals, we followed the same lesioning procedures and applied repeated electrical stimulation via an ECoG electrode 8 mm away from the lesion center. We assessed the neural activity changes within the sensorimotor cortex as quantified by ECoG signal power. In addition, we performed histological analysis that consists of Nissl staining with thionin and immunostaining with antibodies against NeuN and two common plasticity markers, postsynaptic density-95 (PSD-95) and growth-associated protein-43 (GAP-43). Our ECoG signals showed decreased power at the lesioned site and reduced power at all sites post-stimulation. Given that Nissl staining revealed a smaller lesion volume following stimulation, this suggests that stimulation may exert a neuroprotective effect by reducing energy consumption. In addition, we observed strong immunoreactivity of PSD-95 and GAP-43 outside the lesion boundary. We will quantify the expression of these two markers in all animals by the number and size of immunoreactive puncta within the sensorimotor cortex using confocal microscopy to study the level of stimulation-induced plasticity. These results will shed light on the mechanisms governing neuroplasticity following injury and subsequent stimulation in NHPs. With the similarity between NHP and human brains, it paves the path for developing effective stimulation-based therapy for stroke rehabilitation in clinical studies.

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Poster

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Program #/Poster #: P981.07

Topic: I.08. Methods to Modulate Neural Activity

Support: FDA/CDRH/OSEL/DBP internal funding

Title: Electrical stimulation evoked neuronal activation patterns after chronic cortical microelectrode implantation

Authors: *G. WU, K. SOLARANA, D. X. HAMMER, M. YE;
CDRH/OSEL/DBP, U.S. Food and Drug Admin., Silver Spring, MD

Abstract: Multiple neuromodulation devices have gained FDA-approval for treating a spectrum of neurological and psychiatric disorders in the past decade. These devices deliver electrical stimulation below the safety threshold level determined by animal and computational studies on macroelectrodes, for example, a threshold charge density of 30 $\mu C/cm^2$. Novel technology allows fabrication of multi-channel microelectrodes with geometric surface areas (GSA) much less than 2000 μm^2. The safe level of electrical stimulation from these electrodes must be re-evaluated before their therapeutic effects can be investigated in human clinical trials. In this study, we chronically implanted 16-channel microelectrode arrays (GSA of 177 μm^2 per individual electrode) in the motor/somatosensory cortices of transgenic mice (Thy1-GCaMP6s), and used a combination of optical coherence tomography, two-photon calcium imaging, and extracellular recording to evaluate the stimulation effect for activating layer 2/3 neurons. Monopolar stimulation was delivered at various levels of charge/phase, with cathodic-first biphasic currents, at 50 Hz. This multimodal approach allowed us to examine the relationship between stimulation levels and the activity patterns of neurons proximal or distal to the stimulation electrode. Three patterns of neuronal activation were observed, sustained calcium signals throughout the stimulation period, transient calcium activity at the initiation of stimulation, and rebound neuronal activity after the termination of the stimulation. Our results showed a reduction in the electrical threshold to evoke calcium activity from 7 days post-implantation to 21 days post-implantation. In addition, neurons distal to the electrode appeared more responsive to electrical stimulation compared to neurons in the proximal field. It suggests that the neurons near the electrodes remain suppressed or damaged from the penetrating electrodes or by very low stimulation. Further study is needed to investigate whether neurons distal to the electrodes are activated directly by the stimulation electrical field, or indirectly via collateral axons of neurons near the electrode. Our result indicates that the change of neurostimulation threshold needs to be scrutinized during and after the early recovery phase to avoid overstimulation and excitotoxicity.

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Poster

P981. Electrical Methods to Modulate Neural Activity V

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Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant R21MH107671
NSF Grant 1631329

Title: A user-friendly, universal closed-loop phase-locked stimulation controller based on oscillatory episodes in the LFP

Authors: *J. DITTERICH;
Univ. of California, Davis, CA

Abstract: Synchronized oscillatory activity has been proposed to play important roles in modulating functional connectivity between populations of neurons and in memory storage/retrieval processes. Testing these hypotheses requires techniques that allow manipulation of the timing relationship between neural activity in different brain areas. Closed-loop phase-locked stimulation is such a technique. We have recently published a user-friendly, universal, robust algorithm for the detection of oscillatory activity in the LFP and prediction of its phase. Oscillatory episodes are detected based on analyzing the short-time power spectrum. An adjustable zero-phase bandpass filter in combination with the Hilbert transform are used for phase estimation. Linear extrapolation based on a Bayesian frequency estimate is used for phase prediction. Here we present a real-time implementation of this algorithm on an embedded device, which serves as a user-friendly, universal, standalone closed-loop phase-locked stimulation controller. The device is provided with an analog LFP signal, which is sampled at 1 kHz, and outputs a digital control signal for gating a stimulator. It can therefore be used with virtually any recording system and any stimulator. The algorithm was optimized for real-time implementation by performing the bandpass filtering in the frequency domain instead of the time domain, which reduces the number of required spectral transformations, and by replacing one of the time-consuming robust linear regressions with a standard linear regression after artifact rejection, which did not impact the overall detection and prediction performance. The embedded device features an FPGA, which is used for the time-critical input and output operations as well as a coprocessor for the spectral transformations, and a dual-core ARM processor running a real-time Linux operating system, which handles the remaining aspects of the algorithm and the user interface. Only a few milliseconds are needed between retrieving the last LFP sample of an analysis window and scheduling the time intervals, when the stimulator will be activated. The only primary parameters that have to be specified by the user are the range of frequencies, within which oscillations are to be tracked, and the phase range, during which the stimulator should be active. Other parameters, like frequency-dependent detection thresholds for the presence of

oscillatory activity and filter parameters, are adjusted automatically by the algorithm, which makes the device easy to use.

Disclosures: J. Ditterich: None.

Poster

P073. Adolescent Development--Animal Models II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P073.01

Topic: A.09. Adolescent Development

Title: Intermittent access to sucrose for rat pups: the short and longer-term effects

Authors: *G. SENTHINATHAN[1], R. EIKELBOOM[2];
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Abstract: Availability can have profound influence on the consumption of foods and drinks. The 2-phase intermittent-continuous protocol (ICP) examines sucrose solution intake in two groups of rats and finds intermittent access (24h out of every 72h) significantly increases intake (Eikelboom & Hewitt, 2016). In Phase I, groups receive intermittent or continuous access to a 4% sucrose solution, and with adults this results in a long-term elevation (a doubling) in the intermittent group. In Phase II the groups are shifted to a common alternate-day sucrose schedule and the difference is maintained. Adult rats given 16% sucrose in Phase I do not differ in consumption, but in Phase II with 4% sucrose, an unexpressed elevation in the intermittent rats becomes evident. Other work suggested that pups do not show the ICP associated intake elevation (Senthinathan & Eikelboom, 2013). In four parallel experiments using the ICP, male Sprague-Dawley pups were randomly assigned to every day (ED) or every third day (E3D) access to sucrose in Phase I for 16 days (22-37 days of age). In Exp. 1 the groups were given 4% sucrose in Phase I, and in subsequent experiments the groups were given 16% sucrose in Phase I. In all experiments, rats were given alternate-day access to 4% sucrose in Phase II (starting at 39 days old). No consumption differences were evident in Phase I with either 4 or 16% solutions. In Exp. 1 no differences between groups were evident in Phase II but in Exp. 2 & 3 the intermittent rats showed a gradual elevation in consumption in mid to late adolescence relative to the continuous rats even though both groups were now on the same alternate day schedule. In Exp. 4, pups were tested with the ICP and 16% in Phase I, followed by a 10-day gap without sucrose for some groups (+Gap groups) between Phase I-II to examine the robustness of developing sucrose intake differences. A robust sucrose intake difference slowly emerged in Phase II, with the gap itself inducing an additional elevation in consumption (an elation effect) that was independent of the intermittent vs. continuous difference. Sucrose availability with more concentrated solutions, while not showing an immediate difference, can have a profound delayed

influence on pups as the potentially maladaptive effects of intermittent sucrose access remains latent until later in life.

Disclosures: G. Senthinathan: None. **R. Eikelboom:** None.

Poster

P073. Adolescent Development--Animal Models II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P073.02

Topic: A.09. Adolescent Development

Title: Effects of Consumption of a Western Diet and Physical Exercise Before and During Pregnancy in Adult Female CD1 Mice.

Authors: *S. ROSALES-HERRERA[1], M. ORTIZ VALLADARES[2], R. PEDRAZA-MEDINA[2], J. GUZMÁN-MUÑIZ[2], N. MOY-LÓPEZ[2];
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Abstract: One of the main health problems in the world is obesity, it defines itself as an increase in fat and is characterized by unleashing effects that can be harmful to health. There are factors that influence this pathology, among those, genetic and environment factors. Gestation is a vulnerable period for habit modification that can affect the mother and offspring's health. This precise period demands great physical and psychological changes, which together with social demands can affect optimal development during this stage, exposing various pathologies including obesity. The World Health Organization mentioned that in the world more than 650 million adults between 18 and older ages are obese individuals. Recent scientific research shows that some of the causes of this suffering are related to lifestyle modifications, these involve bad dieting life habits and low or even non-existing physical activity; therefore, focusing the attention on these styles can be the key not only to solve the problem but to prevent it. Know the effects of western diet (WD) and physical exercise (PE) before and during adult female CD-1 mice gestation. Four groups of females were established and classified as follows: Group 1 WD with PE, Group 2 WD without PE, Group 3 Balanced diet (BD) with PE, and Group 4 BD without PE. The PE consisted of 1 hour on a treadmill at a speed of 10m / min, from Monday to Friday, 4 weeks before gestation, then during the first two weeks of gestation. The WD administration started from gestation day 1 (G1) until postnatal day 7 (PN7). We made a weekly record of weight and a daily record of food and drink (water) consumption before and during pregnancy. The results showed that the PE during pregnancy helps to prevent excessive gain when accompanied by a DB, however, speaking of a WD, there is a trend of weight gain compared to those females that had a DB without PE. The WD + PE mothers had a higher weight compared to the other groups; however, this may be due to the increase in muscle mass from physical activity rather than from the effect of diet alone. On the other hand, it was

observed that PE does not modify the intake of a DB during pregnancy, but it does increase the consumption of WD. Regarding caloric consumption with WD, it was higher regardless of whether he does PE. In turn, a decrease in water consumption was observed in mothers with WD, as it was a food with higher humidity compared to regular food. A BD in conjunction with PE becomes important for the prevention of excessive weight gain, thus contributing to metabolic disorders that may be detrimental to health in the future. Optimal health before and during gestation also ensures the physical and mental development of the offspring.

Disclosures: S. Rosales-Herrera: None. **M. Ortiz Valladares:** None. **R. Pedraza-Medina:** None. **J. Guzmán-Muñiz:** None. **N. Moy-López:** None.

Poster

P073. Adolescent Development--Animal Models II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P073.03

Topic: A.09. Adolescent Development

Title: Adolescent High Fat Diet Exposure Influences Microglial Activation in the Frontal Cortex

Authors: *A. B. DAVIS, K. R. LLOYD, T. M. REYES;
Pharmacol. & Systems Physiol., Univ. of Cincinnati, Cincinnati, OH

Abstract: Both obesity and high fat diets (HFD) have been associated with an increase in pro-inflammatory gene expression within the brain. Microglia play an important role in early brain development and may be responsive to HFD. We hypothesized that HFD during adolescence would increase proinflammatory microglial gene expression at baseline, and potentiate the response to an immune challenge (lipopolysaccharide (LPS), IP). From 3-7 weeks of age, male and female mice were fed standard chow (SC) (20% energy from fat) or HFD (60% energy from fat). One hour before sacrifice, mice were randomly assigned to either LPS or saline. Microglia were extracted using a Percoll density gradient and fluorescence-activated cell sorting (FACS) from the frontal cortex of the brain, and pro-inflammatory gene expression was examined. Results will be discussed in the context for early life high fat diet to affect brain development through functional changes in microglia.

Disclosures: A.B. Davis: None. **K.R. Lloyd:** None. **T.M. Reyes:** None.

Poster

P073. Adolescent Development--Animal Models II

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Program #/Poster #: P073.04

Topic: A.09. Adolescent Development

Support: Neurobiology of Adolescent Drinking in Adulthood (NADIA) Grant U01 AA019972

Title: Ethanol-induced conditioned taste aversion and neural activation in adult male rats following adolescent intermittent ethanol exposure

Authors: *J. K. GORE-LANGTON, L. P. SPEAR, E. I. VARLINSKAYA, D. F. WERNER; Psychology, Binghamton Univ., Binghamton, NY

Abstract: Alcohol use is common during adolescence, with binge drinking being the most prevalent pattern of drinking at this age. Adolescent intermittent ethanol (AIE) exposure is a frequently used rodent model of human adolescent binge drinking. One consequence of AIE exposure is reduced sensitivity to the aversive effects of ethanol (EtOH) in adulthood commonly assessed using a conditioned taste aversion (CTA) paradigm. The present study was designed to determine if AIE exposure [4 g/kg, intragastrically, every other day, from postnatal day (P) P25 to P45) in male c-Fos LacZ+ transgenic Sprague-Dawley rats would result in attenuated EtOH-induced CTA and alter neuronal activation in CTA-processing brain regions in adulthood relative to water-exposed controls. On P70, animals were given 1h access to a novel solution (0.9% NaCl) in a new cage, followed immediately by an intraperitoneal injection of a single dose of EtOH (20%) (0, 1.5, or 2.5 g/kg). Testing for EtOH-induced CTA occurred on P70 when animals were again given 1h access to the NaCl solution. with brains collected immediately after the test for ß-galactosidase (proxy for c-Fos) quantification in CTA-associated regions of interest (ROIs) including the prelimbic cortex (PrL), infralimbic cortex (IL), dorsal and ventral subdivisions of the agranular insula (AID and AIV), nucleus accumbens core (NAcC), basolateral and central amygdala (BLA and CeA). AIE attenuated EtOH-induced CTA, with AIE males only developing CTA at the 2.5 g/kg EtOH dose and water-exposed controls demonstrating CTA at both EtOH doses. In general, neural activation of the ROIs did not differ as a function of adolescent exposure, with significant decreases of ß-gal expression relative to saline controls evident in the IL of animals conditioned with 1.5 or 2.5 g/kg ethanol as well as in the AIV of rats conditioned with the 2.5 g/kg EtOH dose. The linear regression analyses showed that intake of the NaCl on test day was a significant predictor of ß-gal expression in almost all ROIs. Functional connectivity was assessed via Pearson bivariate correlations (pairwise comparisons of ß-gal+ cell counts in each ROI). Water-exposed controls had significant connections between the AIV, PrL, IL, and NAcC that were not evident in AIE exposed rats. AIE exposure may reduce functional connectivity between brain regions implicated in aversion, with this reduced functional connectivity contributing to relative insensitivity of AIE exposed males to EtOH-induced CTA.

Disclosures: J.K. Gore-Langton: None. **L.P. Spear:** None. **E.I. Varlinskaya:** None. **D.F. Werner:** None.

Poster

P073. Adolescent Development--Animal Models II

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Program #/Poster #: P073.05

Topic: A.09. Adolescent Development

Support: CIHR Grant PJT-15958
NSERC

Title: Cognitive impairments induced by chronic delta-9-tetrahydrocannabinol exposure during adolescence are prevented by concomitant N-acetylcysteine supplementation

Authors: *H. J. SZKUDLAREK, K. B. WIECZERZAK, R. S. MANN, T. C. UZUNESER, M. DE FELICE, S. SUSANNE, B. L. ALLMAN, S. R. LAVIOLETTE;
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Abstract: Cannabis use during critical developmental periods in adolescence can cause long-lasting cognitive impairments and schizophrenia-like symptoms that extend into adulthood. Previous research has demonstrated that chronic delta-9-tetrahydrocannabinol (THC) treatment during adolescence causes pronounced deficits in memory tasks and sensorimotor gating and persistent anxiety symptoms in adulthood. Moreover, changes in intracellular signaling pathways and disruptions in neuronal communication manifesting as increased VTA dopaminergic activity, excessive neuronal firing and abnormal high frequency oscillatory patterns in the prefrontal cortex (PFC) are also observed. As more jurisdictions move towards full legalization of cannabis there is an increased need for developing effective prevention strategies against these deleterious side-effects. Multiple studies point to the role of oxidative stress in the pathophysiology of schizophrenia and antioxidant treatments have proven to be effective in preventing the development of cognitive symptoms in multiple animal models of psychosis. In the present study we explored the potential efficacy of antioxidant treatment in preventing deteriorating effects of adolescent THC treatment on cognitive function in rats, using N-Acetylcysteine (NAC). Adolescent male Sprague Dawley rats (Postnatal day (PND) 35-45) were given twice daily intraperitoneal infusion of either Veh or THC (PND35-37: 2.5 mg/kg; PND38-41: 5mg/kg; PND42-45: 10mg/kg) and a subset of animals from each group received NAC treatment in drinking water (900mg/L; PND35-65). Behavioral and electrophysiological procedures commenced at adulthood (starting at PND75). We demonstrate that rats with adolescent THC treatment displayed significantly reduced recognition memory scores in object recognition tests, have impaired reversal memory and deficits in paired-pulse startle responses. Moreover, electrophysiological recordings in freely moving animals showed that the rats that underwent adolescent treatment with THC displayed increased power of gamma oscillations accompanied with decreased power of delta oscillations in the PFC. Interestingly, NAC

treatment fully prevented THC-induced behavioral impairments and was able to rescue abnormal delta and gamma cortical oscillation patterns. These data suggest that adolescent THC treatment might increase oxidative stress and that dietary antioxidant supplementation may be a safe and effective strategy in preventing pathophysiological changes associated with aberrant adult PFC function following chronic adolescent cannabis exposure.

Disclosures: H.J. Szkudlarek: None. **K.B. Wieczerzak:** None. **R.S. Mann:** None. **T.C. Uzuneser:** None. **M. De Felice:** None. **S. Susanne:** None. **B.L. Allman:** None. **S.R. Laviolette:** None.

Poster

P073. Adolescent Development--Animal Models II

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Program #/Poster #: P073.06

Topic: A.09. Adolescent Development

Title: Sex-specific effects of late adolescent morphine exposure on adult thermal nociception

Authors: *J. R. BOURGEOIS[1], A. M. KOPEC[2];
[1]The Ctr. for Neuropharm. and Neurosci., [2]Neurosci. & Exptl. Therapeut., Albany Med. Col., Albany, NY

Abstract: While often studied in the context of reward circuitry, the nucleus accumbens (NAc) also plays an important role in nociceptive processing. Microglial-mediated refinement of synaptic circuitry in the NAc during adolescence is a necessary component of healthy neurodevelopment, and sex differences in the timing of NAc synaptic pruning have been documented. The opioid morphine can directly activate microglia, and thus may lead to abnormal microglia-mediated neural and behavioral development. We hypothesized that exposure to morphine during sex-dependent critical periods of NAc synaptic development would lead to sex-specific changes in analgesic response to morphine in adulthood. To test this, we treated male and female rats with morphine or saline during late adolescence (P55-59), a time period in which female, but not male, microglial activity is high in the NAc. We subsequently performed a thermal gradient test to determine their preference between a cold zone (10° C), a hot zone (47° C), and an ambient zone in adulthood. No change in baseline temperature preference was observed in either sex following late adolescent morphine exposure. However, an acute 1.5 mg/kg morphine injection resulted in an aversion to the cold zone in the late adolescent morphine-treated female, but not male, rats. Neither adolescent treatment nor sex affected mechanosensation. Together, these data suggest that modality-specific nociceptive circuit refinement is occurring in a sex-dependent manner in late adolescent rats. We are now determining whether morphine alters microglial signaling in the NAc to cause female-specific nociceptive responding in adulthood.

Disclosures: J.R. Bourgeois: None. **A.M. Kopec:** None.

Poster

P073. Adolescent Development--Animal Models II

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Program #/Poster #: P073.07

Topic: A.09. Adolescent Development

Support: AA017823
AA019972

Title: Ethanol-induced neuronal activation in adolescent rats: Impact of age, sex, and prior history of ethanol exposure

Authors: *H. J. COLEMAN[1], T. T. TOWNER[1], K. M. PAPASTRAT[3], S. VILLARREAL[1], E. I. VARLINSKAYA[1], D. F. WERNER[2];
[1]Psychology, [2]Psychology and Behavioral Neurosci., Binghamton Univ., Binghamton, NY;
[3]Univ. of Maryland Sch. of Med., Matyland, MD

Abstract: Adolescence is characterized by a gradual maturation of the brain and is a period when alcohol use is often initiated. Adolescents drink more per occasion than adults, often in the form of binge drinking. Adults with a history of heavy alcohol use in adolescence tend to exhibit deficits in cognitive control. Similar alterations have been reported in rodent models of adolescent intermittent ethanol (AIE) exposure, with some sex-specific effects of AIE recently being identified. AIE exposure also alters sensitivity to ethanol in adulthood, blunting ethanol-induced activation of the medial prefrontal cortex (mPFC) indexed via c-Fos expression. However, c-Fos expression in the PFC during AIE as well as at different adolescent periods has not been investigated. The present study was designed to assess patterns of neuronal activation of the prelimbic (PRL) and infralimbic (IL) regions of the mPFC in adolescent male and female rats in response to ethanol. Experimental subjects male and female transgenic cFos-LacZ rats were acutely challenged with either 0 (water) or 4 g/kg ethanol given via intragastric gavage (IG) on postnatal day (P) 25 or P45. In addition, P45 rats with a previous history of AIE (10 prior exposures to 0 or 4 g/kg ethanol given IG every other day from P25) were included as a chronic exposure condition. As a marker for neuronal activation, we measured β-galactosidase (β-gal) which is a proxy for c-Fos expression. In general, β-gal expression was not affected by ethanol challenge, with a significantly higher number of β-gal+ cells seen following both acute water and ethanol IG administration relative to home cage controls. In females, but not males, activation of the PRL significantly increased with age. In males, however, activation of the IL by water and ethanol was evident in P45, but not P25 adolescents. Activation of the IL in females did not differ as a function of age, with both ages demonstrating significant increases in β-gal expression following IG water and ethanol relative to home cage controls. Chronic exposure to both water

and ethanol blunted β-gal expression in the PRL, but not IL, of males when compared to P45 ethanol-naïve subjects, with this effect evident in both PrL and IL of females. These data suggest that the PRL and IL cortices undergo changes throughout the adolescent period that may differ between males and females, implicating both age and sex in responding of these regions to manipulation rather than ethanol. Further examination of subcortical brain regions that mature earlier in adolescence is needed to better identify acute and chronic effects of ethanol during adolescence.

Disclosures: H.J. Coleman: None. **T.T. Towner:** None. **K.M. Papastrat:** None. **S. Villarreal:** None. **E.I. Varlinskaya:** None. **D.F. Werner:** None.

Poster

P073. Adolescent Development--Animal Models II

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Program #/Poster #: P073.08

Topic: A.09. Adolescent Development

Support: HHMI
NIHP50MH112491

Title: Prefrontal cortical development over adolescence in mouse

Authors: *K. MASTRO[1,3,4], W. WANG[4], B. A. STEVENS[2,3,5], B. L. SABATINI[4,5];
[2]Harvard Med. Sch. Neurobio., [1]Boston Children's Hosp., Boston, MA; [3]Broad Inst., Cambridge, MA; [4]Neurobio., Harvard Med. Sch. Dept. of Neurobio., Boston, MA; [5]HHMI, Chevy Chase, MD

Abstract: Adolescence, the transition from juvenile to adulthood, is a developmental period marked by a prolonged structural change in brain circuits that coincides with an increase in cognitive capacity, sensory seeking, and risk-seeking behaviors. Concurrent with these behavioral changes is the neurodevelopment across prefrontal cortex (PFC). From humans to monkeys to rodents, there is evidence for changes across the adolescent window in prefrontal cortical structure, connectivity, and function. While inroads have been made to understand the developmental mechanisms that drive PFC maturation and how the PFC contributes to an array of cognitive tasks, *the connection between the PFC's developmental changes and its impact on circuit dynamics remain unclear*. To understand how risk genes for psychiatric disease and environment shape this developmental trajectory, we must first establish what is neurotypical. Here, we employ a range of anatomic and electrophysiological techniques to map changes PFC inputs in mice. To track the development of excitatory synapses, we performed slice electrophysiology and recorded miniature excitatory postsynaptic currents across weeks of postnatal development. We found that the frequency and amplitude of these excitatory events

undergo a prolonged change until reaching their stable state in adulthood. To understand how this change in excitatory inputs may impact local circuitry, we used electrical stimulation to measure the direct monosynaptic and feedforward inhibition that pyramidal neurons receive at various stages of development. These results revealed a systematic shift in the organization of prefrontal cortex that far extends the traditional window of adolescence. Moreover, we found that the major source of inhibition, parvalbumin-positive (PV) interneurons underwent a delayed and opposite maturation of their synaptic inputs. These data were supported by optogenetic assisted mapping of two major sources of inputs, cortico-cortical and thalamocortical inputs. In addition to the inputs, we assess changes in excitability that parallel the changes in local and long-range circuitry. Together, these results provide a detailed timeline of how the PFC mature across the full adolescent window and provide the baseline for perturbations to genes and environment.

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Poster

P073. Adolescent Development--Animal Models II

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Topic: A.09. Adolescent Development

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Title: Sensitive period for emergence of contextual fear memory persistence in developing mice

Authors: *A. L. LANJEWAR[1], K. L. EAGLESON[2], P. LEVITT[3];
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Abstract: Sensitive periods are times of heightened plasticity, in which developing circuits have optimal opportunity to undergo experience-based refinement that ultimately shape long-term brain function. In contrast to sensory systems, the timing and cellular and molecular regulators for cognitive sensitive periods remain poorly understood. Here, we first focused on determining the age of onset of contextual fear memory persistence capabilities, a cognitive function defined as the ability to retain a fear memory for at least 7 days, in C57Bl/6J male and female mice. Two groups of mice were conditioned in a fear chamber. The ‘shock’ group received a series of foot shocks during a single session, while the ‘no shock’ group, which were littermate controls, received no foot shocks but were in the chamber for the matched amount of time. Seven days

later, mice were returned to the same chamber for testing, at which time freeze responses were recorded over a five-minute interval. The onset of contextual fear memory persistence was considered the earliest age of conditioning that produced a freezing response on testing day in 'shock' mice that was significantly different from 'no shock' mice. We demonstrated that contextual fear memory persistence emerges rapidly during the fourth postnatal week, with no sex differences in age of onset. A separate cohort of mice further show that from the age of onset, these memories last for at least 30 days, indicating the ability to form remote memories. With the onset of contextual fear memory persistence determined, we next addressed potential molecular mechanisms underlying the emergence of this behavior. Previous studies have shown that the temporally regulated receptor tyrosine kinase MET (MET) modulates the pace of synapse maturation and that extending the normal temporal window of MET expression shifts the timing of critical period plasticity for ocular dominance in the visual cortex. To determine if MET plays a similar role in regulating the timing of the emergence of contextual fear memory persistence, *Met* expression was sustained past its normal window of peak expression through expression of a transgene. There was, however, no shift in the onset of contextual fear memory persistence in these mice compared to control genotypes. In conclusion, we have defined precisely the onset of contextual fear memory persistence in C56BL/6J mice and demonstrate that a decline in MET signaling is not necessary for the emergence of this cognitive function. The age of onset of contextual fear memory persistence can be used in future studies to test other candidate regulators that may be responsible for the temporal emergence of this cognitive capability.

Disclosures: A.L. Lanjewar: None. **K.L. Eagleson:** None. **P. Levitt:** None.

Poster

P073. Adolescent Development--Animal Models II

Location: Virtual Only

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Program #/Poster #: P073.10

Topic: A.07. Developmental Disorders

Support: NIH ES029464

Title: A two-hit model of early immune activation in rats reveals sex-dependent alterations in anxiety and sociability behaviors

Authors: *L. K. DAVIS[1], K. SANCHEZ[2], M. BELL[3], L. K. FONKEN[2], A. C. GORE[2]; [1]Univ. of Texas at Austin Dept. of Neurosci., Austin, TX; [2]Div. of Pharmacol. and Toxicology, [3]Univ. of Texas at Austin, Austin, TX

Abstract: Early life represents a time of significant change and growth in many neurological processes; therefore, environmental stressors during this critical period may result in lasting

perturbations in neurological function. We coupled two environmental challenges: the endocrine-disrupting chemical (EDC), polychlorinated biphenyl (PCB), and lipopolysaccharide (LPS), to explore the long-term effects of environmental stressors given during critical periods of development. Rats were exposed to daily PCBs during early perinatal development (embryonic day (E) 8 through postnatal day (P) 4), followed by a single LPS hit prior to puberty on P24. Upon reaching adulthood, rats underwent a battery of affective and social behavioral testing prior to tissue collection for immunohistochemistry and gene expression analyses. We report here that perinatal PCB exposure increased anxiety-like behavior in the elevated plus maze, as measured by increased time spent in the closed arms. A later hit of LPS enhanced these anxiogenic effects. Male rats given PCBs and LPS showed similar anxiety-like behavior in the light dark box, though this effect was not seen in females. Sociability appeared to be bidirectionally affected by EDCs: males exposed to PCBs showed mild enhancement in social preference during the three-chambered sociability test, while females given PCBs showed decreased preference for a same-sex stimulus rat during the same test. Though PCBs alone did not affect social investigation behavior during the juvenile social exploration test, the additional hit of LPS resulted in diminished investigation behavior, although only in males. This is one of the first studies to explore how EDCs may prime the neuroimmune system to a later immune challenge, and our findings are in line with other two hit models of immune activation. Our results lend evidence to the idea that females may be more resilient to early life stressors that may contribute to the appearance of neurodevelopmental disorders such as Autism Spectrum Disorder, a disorder that disproportionately affects men. Future research aims to examine how this two-hit model alters microglial morphology and activation in the hippocampus and prefrontal cortex.

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Poster

P073. Adolescent Development--Animal Models II

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Topic: G.09. Drugs of Abuse and Addiction

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Pearson Center for Alcoholism and Addiction Research

Title: Effects of two PDE10A inhibitors on alcohol self-administration and striatal gene expression in naïve and post-dependent rats

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Abstract: Alcohol use disorder (AUD) is a chronic, relapsing disorder that afflicts 29% of Americans in their lifetime, can be disabling and increases mortality. New drug targets and neurobiological insight for AUD are needed. Compulsive drinking putatively involves a relative underactivity of indirect pathway medium spiny neurons (iMSNs) that subserve adaptive behavioral selection vs. overactive direct pathway MSNs (dMSNs) that drive drinking behaviors. Compulsive drinking also putatively involves a shift to dorsal (caudate-putamen) from ventromedial (nucleus accumbens) striatal control of ethanol-related behavior. We hypothesized that decreasing the activity of phosphodiesterase 10A (PDE10A), an enzyme differentially expressed in striatal MSNs, reduces compulsive drinking. To test our hypothesis, we studied ethanol post-dependent male and female adult Wistar rats in operant fixed-ratio self-administration of ethanol vs. water after 1-hr pretreatment with either MR1916 or AMG579 (i.p., 0, 0.4, 0.2, 0.1 and 0.05 μmol/kg) (n = 10/drug/sex), two PDE10A inhibitors, in a within-subject manner. To avoid nonspecific motor effects, a bar test was performed to identify the minimum dose of each compound that elicits cataleptic-like behavior; doses below that were used for the self-administration studies. In separate rats, we investigated the expression of pathway marker and immediate-early genes of interest (*Pde10a, Drd1, Drd2, Adora2a, Penk, Pdyn, Tac1, Egr1, Fos, Fosb*) in the caudate-putamen and nucleus accumbens of ethanol-naïve and post-dependent, chronic intermittent ethanol vapor-exposed rats (n = 12/sex and 18/drug/sex, respectively). Preliminary results show that equimole doses of AMG579 elicit a stronger state of catalepsy in female compared to male rats. Additionally, MR1916 dose-dependently reduced self-administration of alcohol by post-dependent male rats in relation to whether they were high (>0.6 g/kg) vs. low drinker rats. Collectively, our study sheds light on the neurobiological role of PDE10A in distinct striatal circuits on post-dependent drinking behaviors as well as the potential impact of novel translatable PDE10A inhibitors to treat AUD.

Disclosures: L.B. Bertotto: None. **D. Lampson-Stixrud:** None. **A. Sinha:** None. **E. Sakoulas:** None. **E.P. Zorrilla:** None.

Poster

P073. Adolescent Development--Animal Models II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P073.12

Topic: G.05. Mood Disorders

Support: NIH R21 MH124036-01A
Pearson Center for Alcoholism and Addiction Research
NIH/NIAAA Institutional Training Grant T32 AA007456
National Institutes of Health Clinical Translational Science Award (NIH CTSA)
STSI TL1 Training Program TR002551

Title: Trapping insular cortex ensembles in a mouse operant self-administration model of loss of control eating

Authors: *G. MACEDO[1], S. BAGSIC[1], A. KREISLER[1], B. HUI[2], J. HUANG[2], C. WILLIAMS[2], E. ZORRILLA[1];
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Abstract: Loss of control (LOC) eating, the sense of being unable to control one's intake, is a hallmark of binge eating disorders. Many models of binge-like eating have been developed that study overeating within a brief time; however, less work has examined the substrate underlying loss of control, modeled as eating compulsively persisting despite adverse consequences or incorrect outcomes. The anterior insular cortex (AIC) subserves visceral-emotional functions, including taste processing, and is implicated in drug craving and relapse. The AIC sends inputs to the nucleus accumbens core (AcbC), which subserves reward and reinforcement. AIC-AcbC projections have been implicated in compulsive-like drinking in a model of alcohol addiction and in compulsive-like food self-administration in our model of binge eating. Previous data in our laboratory showed that rats with intermittent access to a preferred palatable (P) food developed increased PR breakpoints and footshock punishment-resistant FR food self-administration, validating this as a model of compulsive consumption. Here, we have adapted this model to C57BL/6J mice and are applying it for neuronal activity-dependent analysis. Male and female mice were allowed to acquire fixed-ratio (FR1, FR2, FR3 and finally FR5) 5TUM chow self-administration over 2 weeks via nosepoke responses in daily sessions at dark onset. Matched for weight, daily chow intake, and baseline FR5 self-administration, mice then were assigned to 1 of 3 groups: *ad lib* chow (C), *ad-lib* Palatable food (P; high-sucrose, chocolate flavored 5TUL)), 3) or intermittent Palatable (INT) mice that received 24 hr access to P on 3 non-consecutive days each week (Monday, Wednesday, Friday), with chow otherwise available. All mice received operant self-administration sessions at dark onset on access days. Over subsequent weeks, INT mice developed cycling food intake and body weight in relation to the availability of P food, reduced latencies to respond for food, increased FR5 self-administration, increased PR breakpoints, and increased responding during extinction, as compared to *ad lib* fed controls. Now, we are using neuronal activity-dependent ribosomal profiling in H2B-TRAP mice to identify AIC ensembles that are activated by binge eating in INT mice that developed substantially increased PR breakpoints. The results inform the role of intermittent access in the etiology and modeling of behavioral LOC for translational studies. Molecular identification of LOC-associated AIC ensembles may identify novel targets and will enable future causal study of specific cell types via combinatorial genetic approaches.

Disclosures: G. Macedo: None. **S. Bagsic:** None. **A. Kreisler:** None. **B. Hui:** None. **J. Huang:** None. **C. Williams:** None. **E. Zorrilla:** None.

Poster

P077. Comparative Anatomy: Development

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Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P077.01

Topic: A.10. Development and Evolution

Support: NIH BRAIN U01-NS108637
Society for Developmental Biology Emerging Research Organisms Grant to DCL
NSF PRFB 1812017 to DMR

Title: Identified peptidergic neurons arise after metamorphosis in the nudibranch mollusc, Berghia stephanieae

Authors: A. SHEEHAN[1], C. A. WHITESEL[2], M. D. RAMIREZ[1], D. C. LYONS[2], *P. S. KATZ[3];
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Abstract: Gastropod molluscs play important roles in neuroscience research because they have large individually identifiable neurons. However, the developmental origins of identified neurons are not known. The nudibranch, *Berghia stephanieae*, is easily raised in the lab, allowing developmental studies to be performed. We observed the development of central ganglia from four larval stages, through metamorphosis, and six juvenile stages using immunohistochemical labeling for small cardioactive peptide (SCP) co-stained with DAPI to determine how the central ganglia grow and add peptidergic neurons. We found that the ganglia formed during the early veliger larval stage. When first detected, the ring ganglia, which consist of the cerebral, pleural, and pedal ganglia, had about 130 neurons. By the first juvenile stage (10 days post-oviposition, DPO), the number of cells increased to about 620. Over the course of juvenile development, the number of neurons continued to increase and some neuronal somata began to increase in size. The first neurons exhibiting SCP-like immunoreactivity appeared in the periphery before gangliogenesis. The first SCP-immunoreactive (SCP-ir) neurons in the ganglia were only on the left side in the early veliger stage. The number of SCP-ir neurons increased from about 10 at the veliger stage to 47 in the early juvenile. This increased further to 62 by juvenile stage 5. The rhinophore ganglia first appeared at metamorphosis and were innervated by a pair of SCP-ir neurons that were also present in the adult. Thus, this identified neuron could be traced to the metamorphic transition. Many SCP-ir neurons appeared between juvenile stages 2 and 3, with the majority of the neurons that were identifiable by soma size and position present by stage 4.

While most SCP-ir neurons appeared early in development, the relative size and position of these cells continued to change through stage 5. The later juvenile stages (4-6) corresponded to the fastest rate of ganglionic growth. Careful analysis of cell counts and patterns throughout development revealed that many identifiable SCP-ir neurons formed before juvenile stage 4 and a notable pair could be traced back to the metamorphic transition. Elucidating the birth of identifiable neurons in the gastropod central nervous system can provide insights into the formation of distinct neuronal circuits seen in the adult and allow for comparisons between species that have homologous identified neurons.

Disclosures: A. Sheehan: None. **C.A. Whitesel:** None. **M.D. Ramirez:** None. **D.C. Lyons:** None. **P.S. Katz:** None.

Poster

P077. Comparative Anatomy: Development

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P077.02

Topic: A.10. Development and Evolution

Title: Connectomics of the developing mouse and primate brain

Authors: *G. WILDENBERG[1], H. LI[2], V. SAMPATHKUMAR[2], A. M. SOROKINA[2], D. PAUKNER[3], N. B. KASTHURI[2];
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Abstract: The cellular substrate of developmental learning is thought to be ‘synaptic pruning’, the idea being that neurons in baby brains establish and receive numerous connections, and over development, connections are pruned, as influenced by experience. This sculpting occurs without significant addition or removal of neurons or birth of new neurons, suggesting that developmental learning is mediated primarily by experience dependent refinements of neuronal connections at large scale. Surprisingly, despite its seeming importance, large gaps remain in our understanding and appreciation of synaptic pruning. To address this gap, we used serial electron microscopy (‘connectomics’) to investigate pruning in the primary visual cortex (V1) in the neonatal and adult brains of both mouse and rhesus macaque, two well-defined model systems of neuroscience. To our surprise, we find that the mouse shows little evidence of net synapse loss over developmental life. We are now investigating the primate to determine whether synaptic pruning over development is a species-specific phenomenon.

Disclosures: G. Wildenberg: None. **H. Li:** None. **V. Sampathkumar:** None. **A.M. Sorokina:** None. **D. Paukner:** None. **N.B. Kasthuri:** None.

Poster

P077. Comparative Anatomy: Development

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Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P077.03

Topic: A.10. Development and Evolution

Support: EU-H2020-MSCA-ITN-812777

Title: Developmental expression of CRF receptors and binding protein in the chicken telencephalon

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Abstract: Stress is a protective mechanism for animal adaptation to internal or external stressors, which aim is to prepare the body to challenging physiological and/or environmental conditions. Upon a stressful event, three major responses are activated: autonomic, endocrine and behavioral responses. The hypothalamus plays an essential role in control of both the autonomic nervous system and the endocrine system by way of the hypothalamo-pituitary-adrenal axis (HPA). Hypothalamic function is in turn regulated by several telencephalic structures, among which the amygdala and the hippocampus play critical roles. In mammals, neurons expressing corticotropin-releasing factor (CRF) play a major role in regulating the stress response, but very little is known on CRF pathways in chicken. Knowledge on the expression of receptors throughout development can help to identify brain areas playing a role in the stress response, and can provide insights into neural pathways behind the aversive effects of early-life stress. In this project we focused on the developmental expression of evolutionarily conserved CRF receptors and binding protein. We performed chromogenic *in situ hybridization* in chicken embryonic brains (E8-E18) and hatchlings (P0) for the CRF receptors 1 (CRF-R1) and 2 (CRF-R2), and CRF-binding protein (CRF-BP). Our results demonstrate that, from early stages (E8), CRF-R1 and CRF-R2 are moderate- to highly expressed in many parts of the pallium, as follows: CRF-R1 expression is high in the hippocampus, most subdivisions of the dorsal ventricular ridge, and septohippocampal area, and moderate in the piriform cortex (among other areas); CRF-R2 expression is high in the hippocampus, mesopallium and nucleus basorostralis of nidopallium. In contrast, at E8 CRF receptors show low or negligible expression in the subpallium. At this age, CRF-BP, which buffers extracellular CRF and weakens transmission, is only present in the developing striatum. These general expression patterns remain similar or show small modifications during subsequent stages. For example, at E18 receptors expression in the hippocampus decreases a bit or becomes restricted to specific subdivisions, while they are observed in parts of the central extended amygdala (peri-intrapeduncular island field and oval central nucleus). However, at P0, expression of CRF receptors and binding protein is mostly

negligible in the lateral bed nucleus of the stria terminalis. Overall, our findings suggest that, untill P0, telencephalic CRF transmission occurs mainly in the pallium and open the venue to further studying the role of CRF receptors in the stress response throughout development in chicken.

Disclosures: A.H. Metwalli: None. **A. Pross:** None. **A. Abellan:** None. **L. Medina:** None.

Poster

P077. Comparative Anatomy: Development

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P077.04

Topic: A.10. Development and Evolution

Title: Fluctuating brain asymmetry: A tool for studying the evolutionary developmental ecology (Evo-Devo) of early trauma, maltreatment, and neglect

Authors: *S. B. SERAPHIN;
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Abstract: Studies of human neuroanatomy have advanced from the pseudoscience of phrenology, to the localization of functions with ablation research, and recently culminated in studies of brain wiring or the “connectome”. Future innovations in brain science depend on some departure from the typical laser focus on disease models, and the adoption of holistic approaches for understanding the individual variation in brain anatomy and function that emerge from complex gene-environment interactions or epigenetic processes. In this regard, an emphasis on evolutionary developmental ecology (Evo-Devo) has the potential to integrate cross-species comparisons of neuroanatomy with studies of developmental plasticity. For example, fluctuating brain and body asymmetries have been linked to developmental instability, such as adverse childhood experiences. Using sample data from the published literature, including the new analysis of cortical surface area, volume and thickness data from a well characterized sample of 202 maltreated and 131 control human magnetic resonance imaging (MRI) subjects, I will explore how the evolutionary biological concept of fluctuating asymmetry can be used to describe the brain footprint of early life. It will be argued that together, tractography, measures of hemispheric asymmetry and qualitative ethnographic methods can be used to capture the heterogenous outcomes of the environmentally-specific developmental responses to early trauma, maltreatment and neglect.

Disclosures: S.B. Seraphin: None.

Poster

P077. Comparative Anatomy: Development

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Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P077.05

Topic: A.10. Development and Evolution

Support: NIH Grant RF1 MH124605-01

Title: Establishing a Common Coordinate Framework for Quantitative Cell Census in Developing Mouse Brains

Authors: *F. A. KRONMAN[1], J. NORWOOD[1], L. PUELLES[2], J. ZHANG[3], L. NG[4], J. C. GEE[5], Y. KIM[6];
[1]Dept. of Neural and Behavioral Sci., Penn State Col. of Med., Hershey, PA; [2]Dept. of Human Anat. and Psychobiology, Univ. Murcia Fac of Med., Murcia, Spain; [3]Dept Radiology, New York Univ. Sch. of Med., New York, NY; [4]Allen Inst. for Brain Sci., Seattle, WA; [5]Dept. of Radiology, Penn Image Computing and Sci. Lab., Univ. of Pennsylvania, Philadelphia, PA; [6]Col. of Medicine, Penn State Univ., Hershey, PA

Abstract: A brain atlas serves as a key spatial framework to co-localize structural and functional data and examine different neuronal and non-neuronal cell types across brain regions. For the adult mouse brain, the Allen Common Coordinate Framework (CCF) with 3D anatomical labels currently serves as the standard atlas resource with which to map and integrate results from different studies. In contrast, only 2D atlases based on histological sections are currently available for the developing mouse brain, significantly limiting our understanding of the spatial and temporal development of cellular architecture of the brain. Further, the lack of developmental CCFs significantly hinders progress on cell type mapping of the developing mouse brain by limiting the reproducibility and integration of data from different studies. To address this deficiency, we are working to generate developmental CCFs with associated ontology and undistorted 3D anatomical labels in key embryonic and postnatal ages. Here, we present our ongoing progress to create atlases at postnatal day (P)14 and 56. We first used Magnetic Resonance Imaging (MRI) to acquire ex vivo in skull distortion-free images of C57BL/6 mice at target ages. Then, we computed a morphological and intensity average of 10 brain samples (5 male, 5 female) at each developmental age to create a population-averaged symmetric MRI template brain. Independently, we used tissue clearing and 3D histological labeling methods combined with light sheet fluorescent microscopy (LSFM) imaging to acquire high-resolution histology images of additional brain samples. We co-registered LSFM images to the MRI templates to create multimodal atlas templates. Simultaneously, we imported existing 2D anatomical labels from the Allen Developing Mouse Brain Atlas into our new template. Using these labels to define the ontology, we are manually creating true 3D anatomical labels for the CCFs based on high-resolution LSFM data that is co-registered to undistorted MRI. Once complete, our developmental CCFs will enable a broad field of scientists to leverage modern brain mapping technologies more effectively in studying the developing mouse brain.

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Poster

P077. Comparative Anatomy: Development

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P077.06

Topic: A.10. Development and Evolution

Title: Volumetric study in neonatal patients with hypoxia in the putamen, pallidum, amygdala regions.

Authors: *K. PÉREZ RODRÍGUEZ[1], O. DURÁN NAVA[1], M. GONZÁLEZ DE LA FUENTE[1], M. SANTILLÁN[2], S. HIDALGO TOBON[1,2];
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Abstract: Title: volumetric study in neonatal patients with hypoxia in the putamen, pallidum, amygdala regions.
Author: Karla1, Olaf1, Manuel1, María Esther *Santillán2*, Silvia Hidalgo1,2
1 Department of Physics Universidad Autonoma Metropolitana Iztapalapa2 Hospital Infantil de Mexico Federico Gomez
Introduction: During the last decades, scientific and technological advances in neonatology have allowed the survival of children up to 23 weeks of gestational age with various health problems that impact their brain development. The volumes generated from different brain structures by magnetic resonance imaging have become an indispensable tool to detect neurodevelopmental abnormalities [1,2,3]. The knowledge of an abnormal volume in brain tissues gives us the possibility to detect diseases such as seizures, dementia, multiple sclerosis, aneurysms, cancer or stroke. [4,5].
Methodology: 5 neonates with hypoxia were studied to obtain 3D-T1 images using a 3 T SIEMENS Skyra with a TR= 2200ms, TE=2.45 ms. Volumes were analyzed in the neuroimaging data analysis software (FreeSurfer) of different brain structures. Results: The difference in volume between the structures of both groups was obtained hypoxia vs [5] healthy. The structures with significant differences between both groups were: caudate lobe (0.11±1.45), putamen (0.068±1.6), pallidum (0.62±4.92), hippocampus (0.48±1.2), and amygdala (0.513±3.47) cm3.
Conclusion: According to the results obtained in the studies, the structural regions of the left hemisphere appear more asymmetric, which can be attributed to the hypoxia suffered by neonates in the studies analyzed in this work. The comparison of the volumes allows us to know exactly the affected brain areas, and the subsequent evolution of neurodevelopment, which is a

very useful tool to guide subsequent therapeutic interventions in a focused way and improve the final results.
[1] M. P. Zamudio et.al, Automatic Segmentation of Brain Magnetic Resonance Images in T1, Guanajuato, 2018.[2] Johnson, E. B, et.al., Protocol. Jove Journal. 2019 [3] Ulises Rodríguez Domínguez, et.al., Segmentation of neonatal brain magnetic resonance images, Center for Research in Mathematics A. C., August 2016. [4] Investigation of Brain Structure in the One-month Infant, DC Dean III, Waisman Center, May 2018.[5] Neuroimage, 2021 Aug 15;237:118113. 2021 May 1.[6] Artif Intell Med. 2016 Oct;73:45-69. doi: 10.1016/j.artmed.2016.09.001. Epub 2016 Sep 30. PMID: 27926381 Review.

Disclosures: K. Pérez Rodríguez: None. **O. Durán Nava:** None. **M. González de la Fuente:** None. **M. Santillán:** None. **S. Hidalgo Tobon:** None.

Poster

P222. Tau: Biochemistry and Physiology

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P222.01

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

Support: NIH Grant NS109284
Roy J. Carver Foundation
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Iowa Neuroscience Institute
Carver College of Medicine

Title: Developmental shifts in tau splicing regulate interaction with 14-3-3 β

Authors: *R. BETTERS[1], A. GOTTSCHALK[1], K. FIOCK[1], M. SMALLEY[1], E. LUHMANN[2], L. RADOSHEVICH[2], M. HEFTI[1];
[1]Pathology, [2]Microbiology and Immunol., Univ. of Iowa, Iowa City, IA

Abstract: Alzheimer’s disease (AD) and other dementias are the leading cause of disability in older individuals, the prevalence of which has more than doubled in the last thirty years. Although recent AD clinical trials suggest that early or presymptomatic treatment with beta-amyloid targeted therapies may be effective in limiting disease progression, the tau protein is thought to be the primary mediator of neurodegeneration in the later stages of disease. Our preliminary data shows that tau is extensively phosphorylated in both AD and, interestingly, the developing human brain. During development, this phosphorylation does not appear to have any adverse effects. We therefore sought to identify mechanisms for the resilience of the fetal brain to tau toxicity. We first used co-immunoprecipitation (Co-IP) with bead-linked anti-tau antibodies followed by liquid chromatography-tandem mass spectrometry (LC-MS^2) to identify

differences between the tau interactome in human fetal and AD brain tissue obtained from the Iowa NeuroBank or the NIH NeuroBioBank. Spectra were identified using standard reverse-decoy technique in MaxQuant and differential expression analysis was done using Perseus. We found that tau interaction with 14-3-3 proteins β, γ and η were significantly upregulated in AD compared to the fetal brain. Expression of these proteins does not vary during development or in AD in publicly available proteomic or transcriptomic data sets, suggesting that the difference in tau interaction is driven by other factors. We followed up on our results in human tissue by validating this interaction in HEK 293T cells by conducting a FLAG-tagged 14-3-3 β Co-IP in the presence of either 4R or 3R tau**.** We found that 14-3-3 β interacts exclusively with the longer (4R) tau isoform, and that this interaction is largely unaffected by the phosphorylation state of the tau protein. We reversed the Co-IP in our HEK system through the pulldown of tau, which similarly showed 14-3-3 β binding activity with 4R tau but not 3R tau. Our findings identify a novel interaction between tau and 14-3-3 β at least partially dependent on the inclusion of the second microtubule repeat domain, potentially altering the conformation of tau's two 14-3-3 binding sites and therefore the kinetics of their interaction. We have shown that this additional repeat is only present in the adult brain, suggesting that the presence of 4R tau may be necessary but not sufficient for tau toxicity in both mixed isoform (AD) and 4R (CBD, PSP) tauopathies, and may explain the relative rarity of 3R tauopathies such as Pick's disease.

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Poster

P222. Tau: Biochemistry and Physiology

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Program #/Poster #: P222.02

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

Support: NIH Grant RF1 AG051085
Owen's Family Foundation
Cure Alzheimer's Fund

Title: Extracellular tau oligomers cause nuclear deformation in neurons

Authors: *X. SUN, G. S. BLOOM;
Univ. of Virginia, Charlottesville, VA

Abstract: Neuronal nuclei are normally quasi-spherical. In Alzheimer's disease (AD) and other tauopathies, though, this structure is interrupted by deep invaginations of the nuclear lamina, a meshwork of intermediate filaments on the inner face of the inner nuclear membrane. The lamina is involved in numerous nuclear activities including chromatin organization, nucleocytoplasmic

trafficking of macromolecules, cell cycle regulation, and apoptosis. However, little is known regarding to the mechanism leading to lamina structure disruption and its pathological consequences in AD. Previous work showed that expression of pathogenically mutated human tau in *Drosophila* neurons causes nuclear lamina invagination (Frost, et al. 2016, *Current Biology*, 26; 129-136). To shed further light on the mechanism, we tested the hypothesis that it could be induced in mammalian neurons by extracellular tau oligomers, which are known to be crucial factors in AD pathogenesis. We used primary mouse neuron cultures exposed to extracellular oligomers of human 2N4R tau. Wild type neurons had lamina invagination within 30 minutes of exposure to tau oligomers, as revealed by immunostaining for lamin B1, a major subunit protein of nuclear lamina. This effect of tau oligomers was found to depend on intracellular tau, because nuclear lamina invaginations were not detected in neurons derived from tau knockout mice, unless they expressed tau by lentiviral transduction. Taken together these results raise the possibility that extracellular tau oligomers trigger nuclear deformation in AD and other tauopathies by a mechanism dependent on intracellular tau. Moreover, the results add to the currently short list of known cell biological effects of extracellular tau oligomers on neurons.

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Poster

P222. Tau: Biochemistry and Physiology

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Program #/Poster #: P222.03

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

Title: Brain-specific transmembrane transport receptor 2 is a novel modulator in tauopathies

Authors: *H. LIU[1,2], C. CHEN[1,2], *N. WANG[1,2], X. YANG[1,2], S. SU[1,2], C. RASTEGAR[1,2], S. KOTHA[1,2], V. L. DAWSON[1,2], T. M. DAWSON[1,2], X. MAO[1,2];
[1]Inst. for Cell Engin., [2]Dept. of Neurol., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: In Alzheimer's disease (AD), the hyperphosphorylation of tau (p-tau) and fibrillar tau inclusions are the major components that lead to the filamentous tau aggregation. This abnormal tau aggregation is the characteristic neuropathological hallmark of tauopathies in AD. At present there is no cure or prevention of the disease pathology, and currently there are no effective therapeutic strategies that directly target tauopathies to rescue the abnormalities and memory deficit. Recent understanding of the disease mechanism leads us to focus in developing new drugs towards slowing down the progression of AD by prevention of abnormal accumulation of p-tau and the clearance of aggregated tau fibrils in the neuron. In our current strategy, we initially employed an in-vitro system to perform a high-throughput screening to determine the specific receptors that bind tau aggregates, and we determined the binding efficiency of these

receptors. During this preliminary screening we over-expressed the receptors in the SH-SY5Y cells with biotin labeled tau aggregates to determine receptor binding. In the study, we found that Brain-specific transmembrane transport receptor 2 (BTTR2) had high binding efficiency to the tau aggregates. We also verified the novel role of BTTR2 in mediating the internalization of tau aggregates in neurons using specific BTTR2 antibodies. The aim of the current study is to explore the role of BTTR2 in the mouse model of AD. BTTR2 knockout ($BTTR2^{(-/-)}$) mice show dementia at 6 months of age, so we choose different time-points to evaluate the functional rescue of tau related abnormalities. Our result shows that there is significant increase in the tau phosphorylation in $BTTR2^{(-/-)}$ mice compared with their littermates. The injection of AAV9-CBA-eGFP-2a-P301Ltau caused increased cell-to-cell transmission of tau and tau phosphorylation in $BTTR2^{(-/-)}$ mice. Altogether, our data strongly support that BTTR2 has a critical role in regulating tauopathy. Considering the downregulation of BTTR2 in aged mice, the upregulation of BTTR2 is becoming a promising way to rescue hyperphosphorylation of tau and further abnormal aggregation in AD.

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Poster

P222. Tau: Biochemistry and Physiology

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Program #/Poster #: P222.04

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

Support: Else-Kroener-Fresenius Stiftung
Koeln Fortune
Studienstiftung des Deutschen Volkes

Title: Domain requirements and mechanisms of axonal Tau sorting

Authors: *M. BELL, S. BACHMANN, J. KLIMEK, H. BREUER, H. ZEMPEL;
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Abstract: In Alzheimer’s disease (AD) and related tauopathies, somatodendritic missorting of the axonal Tau protein is a major pathological hallmark. Previous work suggest a critical role for the axon initial segment (AIS) in maintaining polarized Tau distribution. However, the exact mechanisms of AIS-mediated Tau trafficking, i.e. AIS-specific interaction partners and necessary Tau domains or motifs are not fully understood.We perform sorting analysis using a Tau construct library, EB3-based live-cell imaging and leading-edge proximity labelling techniques in three different neuronal cell models (hiPSC-derived neurons, primary rodent neurons, SH-SY5Y-derived neurons) to elucidate the interplay between Tau and AIS

components during the process of axonal Tau sorting.First results revealed the insufficiency of the Tau N-terminal half to target the axonal compartment. More detailed analyses will help to pinpoint Tau domains or motifs that are necessary and sufficient for Tau sorting.Further, successful Tau sorting in SH-SY5Y-derived neurons question the previously reported importance of Ankyrin-G (ANKG) and tripartite motif-containing protein 46 (TRIM-46) for i) axonal Tau enrichment and ii) microtubule orientation at the AIS. For performing the recently developed TurboID proximity labelling, we will introduce fusion constructs of Tau and the promiscuous biotin ligase BirA into hiPSC-derived neurons via lentiviral delivery. This will enable us to unravel the Tau interactome domain- and sorting-dependent by using truncated or modified Tau in the first step, and conduct compartment- and even AIS-specific Tau interaction studies in the future. Gaining these data would not only improve the understanding of axonal Tau enrichment in healthy neurons, but it would help to understand the detrimental cascades that underlie pathological Tau missorting in AD and other tauopathies.

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Poster

P222. Tau: Biochemistry and Physiology

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P222.05

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

Support: Indiana University Grand Challenge Precision Health Initiative
NIH/NINDS 1R01NS119280

Title: Resistance to Alzheimer's pathology is associated to a decrease in tau seeding activity and pro-inflammatory response

Authors: *N. JURY GARFE[1], Y. YOU[1], J. REDDING[2], H. KARAHAN[1], A. LEE-GOSSELIN[1], J. KIM[1], J. TRONCOSO[2], C. LASAGNA-REEVES[1];
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Abstract: The neuropathologic hallmarks of Alzheimer disease (AD) are Aβ-amyloid deposits, neurofibrillary tangles (NFTs) and the degeneration of vulnerable neurons and their synapses. Asymptomatic AD (AsymAD) is described as the status of subjects with preserved cognition assessed but with identifiable AD pathology at autopsy. Currently there is no clear understanding of the mechanisms involved in this resistance to AD pathology nor even a detail characterization of these AsymAD cases. Therefore, the detail analysis of these cases might prove a significant knowledge to human resilience for AD. Considering that decreased inflammatory response and

the tau pathology seems to be uncoupled from neuron death in AsymAD compared to typical demented AD, we conducted a detailed histological and biochemical analysis using postmortem brain samples from age-matched control, demented AD and AsymAD subjects. Our analysis identified lower levels of phospho-tau species and seeding activity in AsymAD compare to AD. Using subcellular fractionation, we found accumulation of seed-competent tau predominantly in cytosolic compartment in AsymAD samples, while in AD cases the distribution in cytosolic and synaptic fractions was similar. Biochemical measurements revealed differences in total and cytosolic Aβ40 but not Aβ42 in AsymAD compared to AD cases, whereas in the synaptic fractions both Aβ42 and Aβ40 did not change. RNA expression analysis by Nanostring revealed a distinct neuroinflammatory response in AsymAD versus AD patients. Our data suggest that the main differences between non-demented individuals with high loads of AD's pathology and typical demented AD patients lies on the structural diversity of tau aggregates, its ability to reach the synapse and distinct neuroinflammatory response.

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Poster

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Program #/Poster #: P222.06

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

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NIH Grant NS094557
NIH Grant AG055771
NIH Grant AG060718
AHA Grant 17CSA33620007
NIH Grant AG058522

Title: Characterization of biologically active tau oligomeric species

Authors: *R. KAYED[1], U. SENGUPTA[2], F. LO CASCIO[2], N. PUNGUALMALI[2], M. MONTALBANO[2], N. BHATT[2], A. BITTAR[2], C. JEREZ[2];
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Abstract: Tauopathic neurodegenerative diseases, including Alzheimer's disease (AD), are characterized by the appearance of extracellular lesions composed of aggregated and post-translationally modified tau proteins. The resulting tau pathology is an established marker for differential disease diagnosis and staging, a surrogate marker for neurodegeneration, a potential vector for disease propagation, and a source of toxicity in biological models. In comparison to

the well-established methods for isolation/preparation and biochemical and structural characterization of stable tau filaments from tauopathy brain, the characterization of smaller and much dynamic oligomeric tau aggregates has not been investigated to the same degree. As a result, tau oligomers that may associate most closely with disease propagation and toxicity as suggested by a large body of research, are not fully established. Herein, we leverage our well-optimized brain-derived tau oligomers (BDTOs) from well-vetted human tauopathy cases of AD, progressive supranuclear palsy (PSP) and dementia with Lewy bodies (DLB). In addition to our rigorous biochemical characterization of the BDTOs by immunological probing, and structural characterization by proteolysis and microscopic analyses, we have optimized Fluorescent Amyloid Multi Emission Spectra (FLAMES) microscopy for cataloging and comparing tau conformational variants across a wide range of samples, from brain tissue sections and BDTOs to in vitro aggregated tau assemblies. For selective immunodetection of the BDTOs, we have used our novel four fully sequenced and epitope mapped Tau Oligomer Monoclonal Antibodies (TOMAs), which recognize non-continuous sequences on tau. Furthermore, the bioactivity of the BDTOs was assessed by evaluating their seeding propensity in biosensor cells and primary cortical neurons from tauopathy mice and their effects on synaptic function by electrophysiological studies. We observed that the BDTOs differed in their immunological properties, morphology, and sensibility/stability to proteolysis. The BDTOs were distinguished based on FLAMES spectral signature. Distinct seeding propensity and impairment of long-term potentiation (LTP) indicated their differences in bioactivity as well. Our thorough investigation/characterization of biologically active disease-relevant tau oligomers will provide new opportunities to diagnose and evaluate therapeutics beyond the current capabilities for AD and related tauopathies by developing specific tau aggregation inhibitors and/or diagnostic probes for pathological tau protein.

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Poster

P222. Tau: Biochemistry and Physiology

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Program #/Poster #: P222.07

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

Title: Interferon-induced transmembrane protein 3 and Tau in Alzheimer’s Disease

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Abstract: Interferon-induced transmembrane protein 3 and Tau in Alzheimer's Disease Lauren Jonas[1, 2], Georgia Frost[2], Yueming Li[1, 21]Weill Cornell Graduate School of Medical Sciences, Weill Cornell Medicine, New York, NY, 10021, USA, [2]Chemical Biology Program, Memorial Sloan-Kettering Cancer Center, New York, NY, 10021, USA,
Alzheimer's disease (AD) is a neurodegenerative disease characterized by extracellular amyloid plaques and intracellular hyperphosphorylated tau. Recent work highlights Interferon-induced transmembrane protein 3 (IFITM3), an innate immunity protein, as being a key contributor to amyloid-associated AD pathology. Upregulated in post-mortem AD patients and by AD-associated inflammatory cytokines, in vitro and *in vivo* work has shown IFITM3 to play a role in amyloid processing, as well as in the disease-associated immune response. While much remains to be understood on IFITM3's role in amyloid-related pathology, little is known of the relationship between IFITM3 and tau. Current behavioral and biochemical studies indicate that an IFITM3 deficit in the tau (PS19) mouse model contributes to preserved cognitive function, altered hyperphosphorylated tau deposition, and reduced neuronal degeneration. This project aims to elucidate the mechanism through which IFITM3 functions, as current studies highlight IFITM3's critical role in AD, as well as its potential as a novel target for pharmacological intervention.

Disclosures: L. Jonas: None. **Y. Li:** None. **G. Frost:** None.

Poster

P222. Tau: Biochemistry and Physiology

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

Support: VA Merit Review Grant #I01BX004044
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Nancy and Buster Alvord Endowment

Title: TDP-43 promotes pathological tau phosphorylation and selective neurodegeneration

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Abstract: Although the neuropathology of Alzheimer's disease (AD) is classically defined by amyloid β (Aβ) and tau aggregates, accumulating evidence suggests that TDP-43 may also play an important role in AD pathophysiology. The presence of both AD and TDP-43 pathology at

autopsy is associated with more severe cognitive impairment and accelerated cognitive decline; however, the mechanisms underlying TDP-43 toxicity in AD are not currently known, necessitating models of combined pathology. The nematode *C. elegans* has been widely used to study amyloid, tau and TDP-43 proteotoxicity separately, and we have previously shown that co-expression of tau and TDP-43 in the same organism has synergistically deleterious effects. Recently we developed new low-expression models of combined Aβ and TDP-43 proteotoxicity (Aβ+TDP Tg-low) and tau and TDP-43 proteotoxicity (tau+TDP Tg-low) in *C. elegans* to further explore relationships between these pathologic peptides. Our results show that very low levels of TDP-43 are sufficient to enhance tau but not Aβ neurotoxicity, and that synergism between tau and TDP-43 is largely rescued by loss-of-function of the potent tau modifier *Suppressor of Tau Pathology 2 (sut-2)*. We also demonstrate progressive neuronal degeneration in a cell-type dependent manner, suggesting a degree of selective vulnerability to the combined effects of tau and TDP-43. Understanding TDP-43 and tau synergy *in vivo* is critical to ultimately treating mixed pathology AD. Ultimately this model will be useful for identifying additional genetic modifiers of mixed tau and TDP-43 neurotoxicity and aid in a deeper dissection of the biology underlying these phenotypes.

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Poster

P222. Tau: Biochemistry and Physiology

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Program #/Poster #: P222.09

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

Support: The JPB Foundation, Grant 322 & 839 (to SCS)
The Fisher Center for Alzheimer’s Research Foundation
Cure Alzheimer's Fund (to RET)

Title: Oleanolic Acid Carbamates Reduce Abeta Peptides and Phospho-Tau Protein in Alzheimer’s Disease Cell Culture Models

Authors: A. SINHA[1], V. BUSTOS[1], L. QUINTI[2], Y. TIAN[1], C. ECHMALIAN[2], W. LUO[3], N. Y. IP[4], D. KIM[2], R. E. TANZI[2], ***S. C. SINHA**[3,1];
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Abstract: Oleanolic acid (OA), **1**, (**Fig. 1A**), is a pentacyclic triterpene natural product found abundantly in numerous edible and medicinal plants. Both OA and its derivatives are active against several diseases, including Alzheimer's disease (AD)[1]. Previously we have shown that OA benzyl carbamate, **2**, reduces production of Aβ peptides in an AD cell model through inhibiting γ-secretase cleavage of Amyloid precursor protein (APP) and rescues impairment of synaptic plasticity in double transgenic AD mice[2]. We have now tested several new analogs of compound **2**, including OA-(2-pyridine)methylamine carbamate, **3**, and OA-morpholine carbamate, **4** (**Fig. 1A**), using N2a695 mouse neuroblastoma cells expressing human APP or using 3D human neural cell culture assay[3]. The latter model carries familial AD mutations in APP and Presenilin 1 and induces robust extracellular Aβ deposition and high levels of pTau protein in soma and neurites. It provides a unique platform to test effects of a compound on both the Aβ pathology and tauopathy. Here, we report that most OA carbamates reduced production of Aβ peptides in N2a695 cells. Two compounds, **3**and **4**, also reduced Aβ levels and Tau phosphorylation in 3D assay (**Fig. 1B-D**).

References. 1. Ayeleso, T. B.; Matumba, M. G.; Mukwevho, E., Oleanolic Acid and Its Derivatives: Biological Activities and Therapeutic Potential in Chronic Diseases. *Molecules (Basel, Switzerland)* **2017,** *22* (11), 1915. 2. Luo, W.; Ip, F. C. F.; Fu, G.; Cheung, K.; Tian, Y.; Hu, Y.; Sinha, A.; Cheng, E. Y. L.; Wu, X.; Bustos, V.; Greengard, P.; Li, Y.-M.; Sinha, S. C.; Ip, N. Y., A Pentacyclic Triterpene from Ligustrum lucidum Targets γ-Secretase. *ACS Chemical Neuroscience* **2020,** *11* (18), 2827-2835. 3. Choi, S. H.; Kim, Y. H.; Hebisch, M.; Sliwinski, C.; Lee, S.; D'Avanzo, C.; Chen, H.; Hooli, B.; Asselin, C.; Muffat, J.; Klee, J. B.; Zhang, C.; Wainger, B. J.; Peitz, M.; Kovacs, D. M.; Woolf, C. J.; Wagner, S. L.; Tanzi, R. E.; Kim, D. Y., A three-dimensional human neural cell culture model of Alzheimer's disease. *Nature* **2014,** *515* (7526), 274-278.

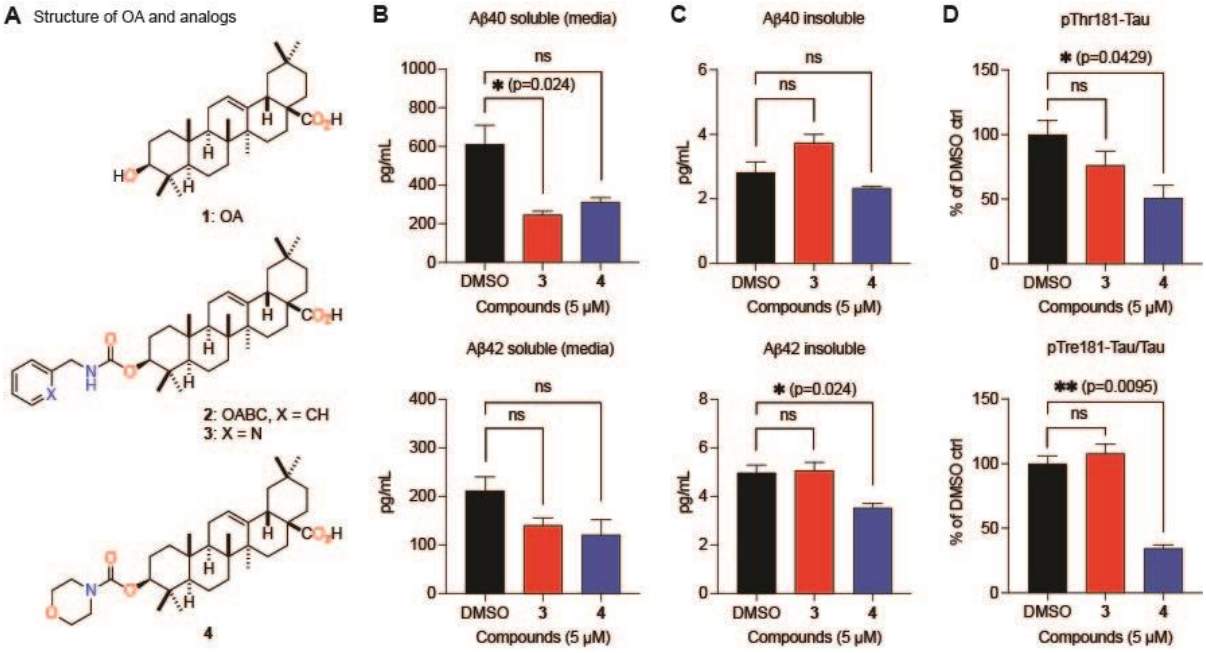


Fig. 1. (A) Structure of OA and carbamates and (B-D) their effects on Aβ production and Tau-phosphorylation determined in 3D human neural cell culture assay.

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Poster

P225. Tau: Preclinical and Clinical Pathology

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P225.01

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

Title: Tau-immunoreactive thorn-shaped astrocytes in brain neurodegeneration and aging

Authors: *A. T. HARRIS[1], L. B. SOLANO[1], J. M. WALKER[1], T. E. RICHARDSON[1], D. W. DICKSON[2], K. F. BIENIEK[1];
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Abstract: Aggregated microtubule associated protein tau in neurofibrillary tangles is a pathological hallmark of Alzheimer's disease (AD). In other tauopathies, such as corticobasal degeneration (CBD) and progressive supranuclear palsy (PSP), astrocytic tau inclusion bodies, astrocytic plaques (AP) and tufted astrocytes (TA), define pathognomonic lesions. New evidence suggests astrocytic tau pathology plays an important role in age-related neurodegeneration (like AD), demonstrated by the recent definition of aging-related tau astrogliopathy (ARTAG). Thorn-shaped astrocytes (TSA) found in ARTAG are also a prominent feature of chronic traumatic encephalopathy (CTE), a mixed tauopathy linked to repetitive head trauma. Understanding similarities and differences of TSA from traumatic and aging etiologies has important ramifications in elucidating disease pathogenesis in CTE and ARTAG. Our goals herein are two-fold: A) identify differences in astrocytic tau proteoforms between these disorders and B) evaluate astrocytic diversity to understand which astrocyte subpopulations are susceptible to developing pathology. To address these goals, immunohistochemistry was performed on postmortem human frontal cortex tissue of CTE (N=4), AD/ARTAG (N=4), PSP (N=2) and CBD (N=2) cases with antibodies against phosphorylated tau (AT8, AT180), N-terminal cleaved tau (C3), 3-repeat and 4-repeat tau (RD3, RD4), as well as astrocytic markers glial fibrillary acidic protein (GFAP), vimentin and complement 3. Color deconvolution digital pathology analysis was employed to quantitatively measure differences between AT8 and AT180. TSA in CTE and AD/ARTAG were negative for C3 and RD3, but positive with AT8, AT180 and RD4. Quantitative analysis revealed equal AT8:AT180 staining in TA and AP, but higher expression of AT8 vs. AT180 in gray matter TSA for both CTE ($p=0.0023$) and AD/ARTAG ($p=0.0006$) by Mann Whitney tests. Similar findings were observed for white matter TSA ($p<0.0001$) combining CTE and ARTAG cases. While astrocytic lesions across all diseases largely

demonstrated GFAP expression, complement 3 was variable between cases. Importantly, vimentin-positive astrocytes were pronounced in CBD and AD/ARTAG but typically absent in PSP and CTE. Gray matter TSA in CTE were rarely immunoreactive for vimentin, yet white matter TSA in ARTAG often exhibited vimentin positivity. Overall, results indicate that while tau proteoforms may be broadly comparable between CTE and ARTAG TSA, differences in astrocytic selective vulnerability could differentiate these pathologic entities. Future astrocytic cell profiling studies will provide further insight on these findings.

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Poster

P225. Tau: Preclinical and Clinical Pathology

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

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Title: Comparison of amyloid and tau pathology in the small and large intestines of individuals with Alzheimer’s disease, Parkinson’s disease, and Dementia with Lewy Bodies

Authors: *A. M. FLODEN[1], G. D. MANOCHA[2], N. I. FROLOV[1], A. E. LERICK[1], C. K. COMBS[1];
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Abstract: Neurodegenerative diseases such as Alzheimer’s disease (AD), Parkinson’s disease (PD) and Dementia with Lewy Bodies (DLB) all present with protein aggregation histopathologies in the central nervous system. In particular, AD is characterized by accumulations of phosphorylated tau in the form of neurofibrillary tangles and Aβ peptide containing plaques. Since enteric neurons of the gastrointestinal system express tau and the amyloid precursor protein, we asked whether phosphorylated tau containing neurofibrillary tangles and Aβ aggregates were present in AD small and large intestines similar to brains. For comparison, we also assessed healthy controls and individuals with PD and DLB. Three male and three female samples were examined from healthy controls, AD, PD, or DLB donors that

ranged in age from 62-95 years. Samples from the mid temporal gyrus (MTG), duodenum, jejunum, ileum, and colon were immunostained for amyloid beta (Aβ) and phosphorylated tau. We employed a semi-quantitative analysis of the immunohistochemistry using a score that ranged from 0 (no staining) to 3 (frequent staining). The MTG served as a positive control for disease pathology. Myenteric and submucosal neurons in both sexes and all groups demonstrated intraneuronal Aβ immunoreactivity with no clear association with AD. However, there was no evidence of extracellular Aβ plaque-like aggregation in contrast to brains. Surprisingly, there was a clear overall increased immunoreactivity in the colons of all groups although there was no increase in AD versus the other groups. Phospho-tau immunoreactivity for p-Ser 199/202 using the AT8 antibody was notably absent from the intestines of all groups in contrast to brains. However, p-Ser 396/404 immunoreactivity, recognized by the PHF-1 antibody, demonstrated myenteric and submucosal staining in both sexes and all intestinal regions of all groups with no clear predominance in AD or one intestinal region versus another. These data indicate that the enteric nervous system appears to be unique from the central nervous system in AD with regard to accumulation of neurofibrillary tangles and amyloid plaques.

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Poster

P225. Tau: Preclinical and Clinical Pathology

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

Support: Tau Consortium (Rainwater charitable foundation)
NIH Grant R01NS095252
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Alzheimer’s Association (NIRG-15-363188)

Title: This Tangle Does Not Exist: Analysis of Neurofibrillary Tangle Morphology via Deep Generative Modeling

Authors: *G. MARX[1], A. MCKENZIE[1], K. FARRELL[1], D. KOENIGSBERG[1], B. RAPOPORT[2], Y. KWON[2], A. COSTA[2], M. SIGNAEVSKY[3], M. PRASTAWA[3], J. ZEINEH[3], G. FERNANDEZ[3], J. CRARY[1];
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Abstract: Background: Neurofibrillary tangles (NFT) are a hallmark histopathological feature of Alzheimer’s disease and other neurodegenerative diseases. NFT undergo a morphological

progression that correlates with disease severity. Improved approaches for measurement and quantification of the evolution of NFT using machine learning would facilitate diagnosis and disease staging.
Methods: We deployed a novel method to analyze NFT morphometry by investigating the latent space of a generative adversarial network (GAN). We trained StyleGAN2 using 537,455 images of immunohistochemically-labeled NFT derived from 526 slides from post-mortem human hippocampal tissue from patients detected using a pre-trained model. Detection of semantically meaningful latent directions was also performed.
Results and Conclusions: Our GAN achieved an Fréchet inception distance score of 8.76. Qualitative assessment of generated NFTs showed the model captured a wide spectrum of NFT morphology. Analysis of the latent space revealed several meaningful latent directions that correlated to tau inclusion density, color, size, and neurite loss. We proposed that when combined, these directions can be used to stage neurofibrillary degeneration.

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Poster

P225. Tau: Preclinical and Clinical Pathology

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Program #/Poster #: P225.05

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

Support: OD-011092
AG-055378
AG-062220
AG-066518

Title: Development of tau pathology in the aged rhesus macaque as compared to humans

Authors: *G. STONEBARGER[1,2], D. I. BROWN[3], R. L. WOLTJER[4], H. F. URBANSKI[1,2], S. G. KOHAMA[5];
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Abstract: During clinical aging, physiological microtubule-associated protein tau becomes hyperphosphorylated in the brain and forms pathological intracellular aggregates. These fibrillous protein structures accumulate in the cytoplasm to become pathological by forming

tangles, which are a hallmark in many neurodegenerative diseases including Alzheimer's disease (AD). While non-AD patients also develop phosphorylated tau (p-tau) tangles in old age, p-tau tangles are correlated with cognitive decline in mid- to late- stage tauopathies. In AD patients, tangles spread from transentorhinal regions to the limbic system, and finally to the isocortex; this system is widely accepted in the clinic as the primary method to determine the advancement of AD postmortem.The rhesus macaque is a widely used laboratory monkey due to its genetic, hormonal, social, and behavioral similarities to humans. Within the last half decade, p-tau tangles have been discovered to arise naturally in very aged rhesus macaques. Herein we used clinical staging in postmortem brains of extremely old rhesus macaques (range: 22.4 - 44.1 years). The entorhinal cortex, hippocampus, and prefrontal cortex were immunohistochemically stained for p-tau in order to encapsulate all three clinical stages and determine whether tauopathy in the rhesus macaque follows a typical spread pattern of AD tangles. Only the very oldest animals showed fully formed neurotoxic tangles, and the few monkeys to reach a tangle load likely sufficient to induce functional alterations were over the age of 39 years—well beyond the average and even previously cited maximum lifespans. This indicates that a primary factor in p-tau detection in this species is age. Most primate researchers do not have monkeys that live to the maximum lifespan, and therefore the phenomenon of natural p-tau was not historically documented. Further, neuronal tangle spread seems to follow an AD-like pattern, but is severely delayed relative to lifespan. However, glial p-tau is the primary tauopathy in this population. While glial tauopathy occurs in aging humans, it is not generally comorbid with AD, suggesting that rhesus macaques—like other nonhuman primates—have pathways to p-tau accumulation which differ from those in AD. These mechanisms may protect from overt dementia, as monkeys do not present with neurodegeneration and dementia, even in the oldest animals. However, deciphering these mechanisms in the monkey model could be key to establishing early clinical interventions into AD, and conversely, clinical data may inform how to best utilize the rhesus macaque model of brain aging to further the AD field.

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Poster

P225. Tau: Preclinical and Clinical Pathology

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

Support: NIH/NIA R01AG069433,
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Title: Exploring the mechanisms of pathological Tau on synaptic vulnerability in Primary Age Related Tauopathy (PART)

Authors: *S. KADAMANGUDI, M. MARCATTI, G. TAGLIALATELA;
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Abstract: In the pathogenesis of aging and dementia, many molecular events converge at synaptic damage or dysfunction. Recent studies demonstrate the detrimental effects of pathological Tau (particularly oligomeric Tau) on synaptic structure and function, thus necessitating further investigation of the mechanisms by which pathological Tau mediates cognitive dysfunction at the synaptic level. Primary Age Related Tauopathy (PART) is a diagnostic term that was coined recently to explain features of a dementia-related illness, characterized by pathological Tau in the absence of amyloid (Aβ) plaque burden. Further, post-mortem studies in PART patients demonstrate the localization of pathological Tau to the medial temporal lobe, avoiding infiltration of the neocortex. Probing the molecular mechanisms which underlie PART thus presents an ideal opportunity to understand the differential effects of Tau (excluding Aβ) on the synapse and study the mechanisms of synaptic resilience in the neocortex. To this end, we sought to compare the levels of (1) toxic oligomeric Tau (TauO) and (2) TauO synaptic binding events between the hippocampus (region with vulnerable synapses) vs. superior medial temporal cortex (SMT; region with resilient synapses). Synaptosomal tissue fractions of the hippocampus and SMT, prepared from 7 post-mortem patients with PART, were used to compare (1) levels of oligomeric Tau by way of Western Blot (Tau5 1^{o}Ab) and (2) number of synapses bound to ('exogenously derived') labeled TauO using size-gated flow cytometry (Guava®, easyCyte™). Results of the Western Blot and flow cytometry analyses revealed no significant difference between the hippocampus and SMT in the amount of toxic oligomeric tau ($p = 0.42$) and synaptic binding of TauO ($p = 0.77$) in PART patients ($n=7$). These results suggest the possibility that, in PART, TauO's cytotoxic effects may lie outside the synaptic interface (i.e. somatic, axonal, or dendritic) or the effects of molecular signaling events downstream of and independent of Tau synaptic binding. These findings should be considered within the context of their limitations, and warrant further investigation (including probes for different Tau isoforms, in different tissue preparations, and immunohistology) and provide a unique opportunity to study the effects of pathological Tau on both synaptic vulnerability and resilience within the brain of individual patients as seen in a singular tauopathy (i.e. PART).

Disclosures: S. Kadamangudi: None. **M. Marcatti:** None. **G. Taglialatela:** None.

Poster

P225. Tau: Preclinical and Clinical Pathology

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P225.07

Topic:

Support: Grant support to JMF from CHSS, GMU
Grant support to ANP and RET from OSCAR, GMU
Grant support to REB from Sigma Xi

Title: Evaluating the efficacy of young blood plasma as a treatment for alzheimer's disease in h-tau mouse models

Authors: *A. N. PERLBERG, K. A. PEDEMONTE, R. E. BARKEY, R. E. TAPP, J. M. FLINN;
George Mason Univ., Fairfax, VA

Abstract: Alzheimer's Disease (AD) is a progressive neurodegenerative disorder that is characterized by the development of tau tangles within neurons, resulting in impairments in cognition and memory. As increased levels of oxidative stress are seen early in the disease and could activate various pathways that result in further AD development, a measure of oxidative stress, superoxide dismutase (SOD), was chosen as the enzyme of interest. In order to counteract these impairments, four-month-old transgenic (Tg, 4510 hTau) and wildtype (Wt) mice were treated with either plasma or saline. Mice were injected with plasma drawn from young, healthy mice over the course of seven treatments. Saline injections acted as a control. To determine if these treatments were effective in reversing AD side effects in the transgenic mice, we performed western blot analysis (n = 4 for each group) to compare concentrations of SOD. Western blot analyses were run with an Oxidative Stress cocktail. Data was analyzed through a two-way ANOVA and analysis of the western blots identified that there were, surprisingly, not significant differences between concentrations of SOD within the brains of Tg mice treated with plasma and the brains of Tg mice treated with saline, as the AD mice did not appear to have existing significantly high measures of oxidative stress. However, plasma injections of the Wt mice showed a significant trend of reduction of SOD when compared to the Wt mice that received saline injections. While plasma injections into Tg mice did not appear to decrease measures of oxidative stress, plasma injections of Wt mice may indicate the potential to use plasma injections to reduce AD pathways and mitigate AD impairments.

Disclosures: A.N. Perlberg: None. **K.A. Pedemonte:** None. **R.E. Barkey:** None. **R.E. Tapp:** None. **J.M. Flinn:** None.

Poster

P225. Tau: Preclinical and Clinical Pathology

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P225.08

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

Support: George Mason University CHSS Grant
Sigma Xi

Title: Young blood plasma treatment reduces neuronal loss and neuroinflammation in h-tau Alzheimer's type mice

Authors: *R. E. BARKEY[1], K. A. PEDEMONTE[2], A. N. PERLBERG[2], R. E. TAPP[2], J. M. FLINN[3];
[1]George Mason Univ., Centreville, VA; [3]Psychology, [2]George Mason Univ., Fairfax, VA

Abstract: This study examined the effects of young blood plasma treatment on inflammation, neuronal loss, and synaptic plasticity in human tau (h-tau) Alzheimer's disease (AD) type mice. AD is defined neurologically by the accumulation of tau tangles and amyloid plaques. AD is also associated with increased neuroinflammation, including chronic microglia and astrocyte activation, neuronal loss, and a down-regulation of synaptic plasticity. Injections of blood plasma from young mice have been shown to reduce the accumulation of neuroinflammation and increase synaptic plasticity and stability in transgenic (tg) AD type mice, as well as rescue behavioral deficits associated with brain pathology. Specifically, CREB and phosphorylated CREB are found to be lowered in AD. Previously, young blood plasma has shown to increase CREB signaling within the hippocampus (HC) in aged mice. Chronic microglia activation may accelerate the progression of neurodegeneration and is reduced in tg mice following young blood plasma injection. In this study, h-tau mice and wildtype mice were injected with either 1.5microliters of young blood plasma or saline 7 times over 21 days. Mice were then assessed for neuronal loss using Cresyl-Violet. The levels of CREB, phosphorylated CREB, brain derived neurotrophic factor (BDNF), and Iba-1 were determined through Western blot analysis. Genotype by treatment interaction effects were found in the infralimbic region of the prefrontal cortex ($p = 0.031$) and in the cortex above the hippocampus ($p = 0.034$) where AD mice treated with saline have significantly lower cell density than all other groups. In these brain regions, AD mice treated with young blood plasma had similar cell density to the wildtype mice. There was also a significant interaction in the white matter density at midline corpus callosum, with the AD saline groups showing significantly lower white matter density than all other groups ($p = 0.014$). Plasma treated mice had significantly higher white matter density at midline corpus callosum than saline treated mice ($p = 0.002$). Plasma treated mice also had significantly higher cell density in the lateral cortex than saline treated mice ($p < 0.001$). Western blot analysis showed a genotype by treatment trend for levels of Iba-1 ($p = 0.10$) with plasma treatment reducing the levels of Iba-1 found in h-tau mice. Western blot analysis showed no differences in the levels of p-CREB, CREB, or BDNF. These results suggest that the prevention of cell loss in AD plasma treated mice may be a result of the reduction of inflammation due to lower levels of chronic microglial activation following plasma injections.

Disclosures: R.E. Barkey: None. **K.A. Pedemonte:** None. **A.N. Perlberg:** None. **R.E. Tapp:** None. **J.M. Flinn:** None.

Poster

P225. Tau: Preclinical and Clinical Pathology

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Program #/Poster #: P225.09

Topic:

Support: GMU CHSS Grant
Sigma Xi

Title: The Effects of Young Blood Plasma Transfusions on Older hTau Mice Modeling Alzheimer's Disease

Authors: *K. A. PEDEMONTE, A. N. PERLBERG, R. E. TAPP, R. E. BARKEY, J. M. FLINN;
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Abstract: Alzheimer’s disease (AD) is a neurodegenerative disorder affecting nearly 6 million individuals in the United States alone. The hallmarks of AD include declines in spatial and episodic memory as well as the build-up of amyloid plaques and tau tangles. In this study, 4-month-old h-Tau P301/CaMKII mice received 7 (150 microliter) saline or plasma injections, taken from young wildtype mice, via intravenous tail injections over 3 weeks. One week after the completion of the injections, the mice were evaluated in Morris Water Maze (MWM) paradigm to measure spatial memory, nesting building to measure activities of daily living and Circadian Rhythm to assess their sleep wake cycles. After completion of the behavioral tasks, the mice were sacrificed, and brain tissue was evaluated using Western Blot and thioflavin-S staining. The amount of hyperphosphorylated tau protein and tau tangles in the mice that received plasma injections were compared to those receiving saline injection. The Morris Water Maze did not find significance across the days, but the 24-hour probe showed that the AD mice injected with plasma found the platform significantly faster than the AD mice injected with saline. It was found that the AD mice injected with plasma built significantly better nests than the AD mice injected with saline. There were no significant findings on Circadian Rhythm. Additionally, Western Blot analysis showed that there was significantly less phosphorylated tau in the AD mice that were injected with plasma when compared to the AD mice injected with saline. There were no significant differences in total tau between the animals. This is in contrast to the Hernandez (2019) findings with similar injections at 8 months old, where there were no behavioral difference but significant differences in Western Blot analysis of phosphorylated tau. These findings indicate that early plasma injections are important in decreasing the amount of hyperphosphorylated tau. This study will provide support for plasma injections as a possible treatment for tau pathology found in AD.

Disclosures: K.A. Pedemonte: None. **A.N. Perlberg:** None. **R.E. Tapp:** None. **R.E. Barkey:** None. **J.M. Flinn:** None.

Poster

P285. Animal Models I

Location: Virtual Only

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Program #/Poster #: P285.01

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: R61NS115102 P51OD011106
T32 GM081061
TL1 TR0023

Title: Tau expression in the brain of normal and hemiparkinsonian rhesus macaques

Authors: *J. C. GAMBARDELLA, W. SCHOEPHOESTER, V. BONDARENKO, J. M. METZGER, M. E. EMBORG;
Univ. of Wisconsin, Madison, WI

Abstract: Tau is a neuronal protein involved in microtubule stabilization and intracellular vesicle transport in axons. In Alzheimer's disease, frontotemporal dementia, and Parkinson's disease, the tau protein becomes hyperphosphorylated and forms pathological aggregates. Rhesus macaques are widely used for studying such neurodegenerative disorders. In this study, we aim to characterize tau expression in the brains of normal (n=2) and hemiparkinsonian (n=3) rhesus macaques (1 female, 4 males; 3.5-6 years; 4.98-7.45 kg). Hemiparkinsonism was induced by administration of a single, unilateral, intracarotid artery injection of MPTP (3 mg/20ml sterile saline). The animals were necropsied 3 months post-MPTP by transcardiac perfusion with heparinized PBS followed by 4% PFA. The brains were extracted and processed for immunohistochemistry (IHC) against tau (HT7, 1:2000) and immunofluorescence (IF) against tau (HT7, 1:100) and β-III tubulin (1:500). Tau protein expression was blindly quantified across 16 brain structures using a semiquantitative rating scale and NIH ImageJ software. The brain regions included prefrontal cortex, anterior cingulate cortex, primary motor cortex, corpus callosum, internal capsule, anterior commissure, caudate, putamen, substantia nigra, globus pallidus external and internal, thalamus, subthalamic nucleus, amygdala, hippocampus, and entorhinal cortex. Tau was ubiquitously expressed in the brains of normal and MPTP-treated monkeys. In both treatment groups, the greatest tau-ir was in the anterior cingulate and entorhinal cortices, while the weakest tau-ir was in the subthalamic nucleus. IHC revealed patterns of intracellular tau-ir across regions with the greatest soma expression in the thalamus and the greatest fiber tau-ir in the substantia nigra. IF colocalization of tau and β-III tubulin confirmed neuronal expression in cell bodies and fibers in all regions. Preliminary comparison of tau-ir in the substantia nigra, globus pallidus, caudate, and putamen of hemiparkinsonian and control subjects suggest nonsignificant differences between groups. Overall, our study is the first to characterize endogenous tau expression in the rhesus macaque brain.

Disclosures: J.C. Gambardella: None. **W. Schoephoester:** None. **V. Bondarenko:** None. **J.M. Metzger:** None. **M.E. Emborg:** None.

Poster

P285. Animal Models I

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Program #/Poster #: P285.02

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: Medical Research Council (R023808)
Biotechnology and Biological Sciences Research Council (R004765)
Wellcome Trust, Sir Henry Dale Fellowship (200501)

Title: Tauopathy causes increased synchronisation of low frequency rhythms and disruption of stimulus-induced gamma in mouse visual cortex

Authors: *F. RIBEIRO RODRIGUES[1], A. PAPANIKOLAOU[1], J. HOLENIEWSKA[1], A. B. SALEEM[1], S. G. SOLOMON[2];
[1]Univ. Col. London, London, United Kingdom; [2]Exptl. Psychology, Univ. Col. London, London, United Kingdom

Abstract: Dysfunction of the microtubule-associated protein tau is a prominent feature of neurodegenerative disorders including dementia. Mutant tau forms hyperphosphorylated aggregates that impair neuronal function and synaptic connections. These impairments should have correlates in the electrical activity in the brain that may facilitate diagnosis and detection. Previous work in mouse models of tauopathy show changes in local field potentials (LFP) in hippocampal and frontal brain areas, but the functional organisation of these areas is poorly understood.
We therefore measured the influence of tauopathy on LFP signals in a sensory brain area (primary visual cortex, V1), in the well-characterised rTg4510 mouse model of tauopathy, at 5 months (early in degeneration) or 8 months (more advanced degeneration). We made chronic LFP measurements from V1 of awake head-fixed male (1) rTg4510 mice, (2) rTg4510 mice in which mutant tau expression was suppressed from the age of 2 months, and (3) wild-type littermates, in the presence and absence of visual stimulation, while monitoring their locomotion and pupil dimension. We used multitaper analysis to measure the power spectra of the LFP in each condition.
We found an overall decrease in gamma power at more advanced stages of tauopathy, that was accompanied by a reduction in stimulus-induced gamma oscillations (ca. 75-85Hz). In addition, we found substantially increased low frequency power (2-10Hz) in both 5m and 8m tauopathic animals during resting state (when animals were stationary, pupils were small, and in the absence of visual stimuli). The increased low frequency power at rest arose when these animals

intermittently entered states of high synchronisation, in which low frequency oscillations were strongly coupled with each other. Mild states of arousal, as indexed by increases in pupil size without locomotion, reduced the likelihood of entering highly synchronised states.
We conclude that during tauopathy, neuronal ensembles in visual cortex enter brief periods of increased low frequency synchronisation, likely reflecting reduced drive to the local network. Reductions in activity within specific high gamma sub-bands may reflect the increased impact of tau dysfunction on functional connectivity within the cortex. Future studies could evaluate if degeneration has distinct impact on different sub-bands of gamma activity in human patients.

Disclosures: F. Ribeiro Rodrigues: None. **A. Papanikolaou:** None. **J. Holeniewska:** None. **A.B. Saleem:** None. **S.G. Solomon:** None.

Poster

P285. Animal Models I

Location: Virtual Only

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Program #/Poster #: P285.03

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: Swiss National Science Foundation number P2ZHP3_191255 (NLA)
Office of Naval Research N00014-13- 1- 0808 and N00014-17- 1- 2737

Title: Bull-headed: tauopathy distribution in relation to traumatic brain injury in muskoxen

Authors: *N. L. ACKERMANS[1], B. WICINSKI[2], T. M. WILLIAMS[4], N. J. GRIMALDI[2], M. VARGHESE[5], J. S. REIDENBERG[3], P. R. HOF[5];
[1]Neurosci., Icahn Sch. of Med. At Mount Sinai,, New York, NY; [2]Neurosci., [3]Anat., Icahn Sch. of Med. at Mount Sinai, New York, NY; [4]Biol., Univ. of California, Santa Cruz, CA; [5]Neurosci., Icahn Sch. of Med. At Mount Sinai, New York, NY

Abstract: Traumatic brain injury (TBI) remains one of the leading causes of death worldwide, with low success of clinical therapy translation from rodent models. Alternative models with extreme behavior may be more representative of the populations most vulnerable to TBI such as athletes and military personnel and offer a new perspective on TBI recovery and prevention. Muskoxen (*Ovibos moschatus*) have thick skulls and large horns, used in headbutting clashes during the seasonal rut. During extended fights, bulls clash heads up to 20 times, at forces around 3000 N. Despite the common belief that their headgear protects their brains from extreme impacts, it has never been investigated experimentally. TBI can be detected in the brain by using histological staining to highlight tau protein, which accumulates in instances of neuronal damage. Our preliminary results indicated TBI-like pathology in these animals in the form of tau-positive histological staining. Brain sections (10 sections, 50 µm thick, each 10 sections apart) from the prefrontal cortex of one male and two female muskoxen were stained by

immunohistochemistry, using the CP13 antibody to detect tau phosphorylated at serine 202. For each specimen, sections were subjected to exhaustive stereological counting, to estimate the density and distribution of tau-positive structures in cortical layers I to VI and white matter. These structures were categorized as neurites, neuropil thread clusters, or neurons stained with fibrillary tau. In all specimens, the highest count of neurites and neuropil thread clusters occurred in layers I-III, and the highest densities occurred in the depths of the sulci. The oldest specimen showed up to four times more tau-positive neuropil threads than the younger specimens, but followed a similar distribution. A concentration of pathological tau in the sulcus depths is a characteristic sign of TBI. In addition, the higher incidence of pathological tau in the superficial brain layers resembles the pattern described for early stages of chronic traumatic encephalopathy (CTE) progression in humans. The repetitive nature of headbutting in not only muskoxen, but bovids in general, presents them as a logical real-world model for instances of CTE and repetitive TBI seen in vulnerable populations such as athletes and military personnel.

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Poster

P285. Animal Models I

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Program #/Poster #: P285.04

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: AG042178
AG047812
NS105473
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AG069333
AG066347

Title: Reduced voltage-dependent anion-selective channel protein 1 protects against phosphorylated Tau-induced mitochondrial dysfunction and synaptic damage in Alzheimer's disease

Authors: *M. VIJAYAN, S. KUMAR, P. JANGAMPALLI ADI, H. MORTON, L. BUNQUIN, H. REDDY;
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Abstract: Abstract: The purpose of our study is to understand the protective effects of reduced levels of mitochondrial outer membrane protein voltage-dependent anion-selective channel protein 1 (VDAC1) against phosphorylated tau (p-Tau) induced mitochondrial abnormalities and

synaptic deficiencies in AD pathogenesis. AD is a progressive nervous disorder that eventually destroys all mental and physical functions. AD affects over 50 million people worldwide, but there is no effective disease-modifying treatment for it. AD is associated with phosphorylated tau, neurofibrillary tangles (NFTs), and extracellular amyloid-beta (Aβ) plaques in regions of the brain that are responsible for learning and memory. Mounting evidence suggests that p-Tau-induced mitochondrial dysfunction and synaptic damage are largely involved in AD progression and pathogenesis. We recently found that abnormal physical interaction between VDAC1 and p-Tau led to excessive mitochondrial fragmentation and reduced mitochondrial fusion. **Methods:** In the current study, we crossed VDAC1 heterozygote knockout (VDAC1+/-) mice +/- mice with transgenic Tau mice and created double mutant (VDAC1+/- X Tau) mice. We studied cognitive behavior (Morris-water maze, Open-Field, Y-maze, and Rotarod), molecular, cellular, ultrastructural changes (mitochondrial number and length) and dendritic spines in 6-month-old WT, VDAC1, Tau and double mutant (VDAC1+/- X Tau) mice. **Results:** We found reduced cognitive decline, reduced mitochondrial fission proteins and also enhanced mitochondrial fusion, mitochondrial biogenesis, autophagy, mitophagy and synaptic proteins and dendritic spines (length and number) in double mutant (VDAC1+/- X Tau) mice relative to Tau mice. Mitochondrial number is reduced and length is increased in VDAC1+/- X Tau mice. **Conclusions:** Our findings suggest that a partial reduction of VDAC1 increased synaptic buttons (Golgi-cox) may potentially ameliorate cognitive function. In addition, reduced p-Tau production in VDAC1+/- X Tau mice eventually reduced mitochondrial dysfunction, maintains mitochondrial dynamics, and enhances mitochondrial biogenesis, synaptic plasticity, autophagy, and mitophagy. Thus, partial reduction of VDAC1 may serve as the therapeutic regime in preventing Alzheimer's disease.

Disclosures: M. Vijayan: None. **S. Kumar:** None. **P. Jangampalli Adi:** None. **H. Morton:** None. **L. Bunquin:** None. **H. Reddy:** None.

Poster

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Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: Alzheimer's Association AARG-18-56663
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Alzheimer's Association AARFD-19-644407

Title: Chop is not a main contributor to tau-mediated toxicity

Authors: *M. CRIADO-MARRERO, L. J. BLAIR;
Univ. of South Florida, Tampa, FL

Abstract: There are many ways cells in the brain can die. In Alzheimer's disease (AD), the accumulation of tau is linked with cell death, but the major cell death pathway remains to be identified. Prior work has shown that pathogenic tau promotes endoplasmic reticulum (ER) stress and subsequent unfolded protein response (UPR) activation. However, the contribution of ER stress-induced cell death to tau-mediated neurotoxicity is unknown. Interestingly, mice lacking CHOP/*Ddit3*, an important regulator of ER stress-induced cell death, have been shown to be protected from ER stress-induced neuronal loss. We hypothesized that silencing CHOP would prevent tau toxicity through the ER stress pathway and reveal the contribution of tau toxicity that is mediated through this pathway. To investigate this, we generated a mouse-targeting CHOP/Ddit3 shRNA AAV9 that also expresses GFP by a separate promoter. We injected shCHOP AAV9 or a shScrambled control AAV9 in the brains of 8-month-old male and female rTg4510 tau transgenic mice and harvested them at 12-months of age, when ER stress is activated, and neuronal loss is occurring in these mice. We performed behavioral studies using the Y-Maze, Open field, and 2-day Radial-Arm water maze task with a reversal paradigm. Unbiased stereology was used to determine neuronal health and the effect of CHOP shRNA on CHOP levels was evaluated by immunofluorescence. We found that AAV9 CHOP shRNA did not alter learning and memory in tau transgenic mice. Evaluation of the tissue revealed a modest, but non-significant increase in neuronal loss from CHOP knockdown, while the dentate gyrus shows a non-significant decrease in neuronal loss. Tissue volume was unchanged in both regions. Very surprising to us, and the opposite of what was expected, we found that in these regions of interest the CHOP levels increased in the animal expressing the CHOP. Overall, this suggests that shCHOP AAV9 does not alter tau-modified behaviors such as learning and memory, particularly at this timepoint. Our results also suggest that there may be a compensatory increase in CHOP signaling in neighboring cells, suggesting that the activation of the UPR in the brains of these mice may be a protective mechanism.

Disclosures: M. Criado-Marrero: None. **L.J. Blair:** None.

Poster

P285. Animal Models I

Location: Virtual Only

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Program #/Poster #: P285.06

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

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Title: Adeno-associated viral gene delivery of wild-type human tau induces progressive neurodegeneration and formation of insoluble tau species in the hippocampi of middle-aged rats

Authors: *G. GABRIELSON[1], C. BURGER[1], N. T. KEHOE[2], R. GORZEK[3], S. OSTING[2], K. R. NASH[4];
[1]Univ. of Wisconsin, Madison, Madison, WI; [2]Univ. of Wisconsin Madison, Madison, WI; [3]Univ. of California-Los Angeles, Los Angeles, CA; [4]Mol. Pharmacol. and Physiol., Univ. of South Florida, Tampa, FL

Abstract: Tauopathies are a broad class of diseases, including Alzheimer's disease (AD), characterized by hyperphosphorylation and aggregation of the microtubule associated protein tau (MAPT). Tau pathology in AD is sporadic and not associated with any known mutation to the MAPT gene. To recapitulate this tauopathy we used bilateral AAV-injections of wild type 0N4R human tau (htau) into the CA1 region of hippocampus of 6-month-old adult male Fischer 344 rats. Controls included AAV coding for green fluorescent protein (AAV-GFP) and uninjected rats. We observed neuronal loss in hippocampal areas CA1 and CA2 at 3-, 8- and 12-weeks post-injection, with significant loss beginning at 8 weeks as measured by Nissl stain, when compared to AAV-GFP or uninjected controls. Immunohistochemical analysis of the hippocampal region of AAV-htau rats using a human specific tau antibody revealed htau expression by 3 weeks and continued at 8 and 12 weeks. Western blot analysis of total tau revealed an elevated but not significant difference in tau expression at 3 weeks, followed by a significant 1.5-fold increases at 8 weeks and 12 weeks. By 8 weeks, AAV-htau animals exhibit hyperphosphorylation of tau at S202, T205, and S396 phosphorylation sites, as measured by western blot analysis. Hippocampal extracts analyzed via sarkosyl fractionation, allowing for separation of tau aggregates into monomeric, oligomeric, and polymeric aggregate species, show aggregation of monomeric and oligomeric tau species by 3 weeks post injection. Our preliminary data indicates aggregation in monomeric and oligomeric fractions (n=2 animals; 4 hemispheres), and polymeric in one of the two rats analyzed at 12 weeks post-injection. No significant behavioral deficits in fear conditioning or Morris water maze were observed at any timepoint, but a trend towards significance was found in cued fear conditioning at 12 weeks post-injection. Investigation of behavioral phenotypes at later time points is currently in progress. In conclusion, the use of AAV-mediated gene delivery of human tau offers a fast and reliable novel approach to generate progressive tauopathy models, which have the advantage of administration at any age to study age as a variable in disease progression. It also may be useful test hypotheses in future studies exploring tau spreading.

Disclosures: G. Gabrielson: None. **C. Burger:** None. **N.T. Kehoe:** None. **R. Gorzek:** None. **S. Osting:** None. **K.R. Nash:** None.

Poster

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Program #/Poster #: P285.07

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: Swedish Research Council
Knut and Alice Wallenberg Foundation
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Swedish Brain Fund
Stratneuro Initiative
Västerbotten County Council

Title: Updates on seeding studies: SOD1 prions transmit aggregation and fatal ALS-like disease - Introducing Strain C

Authors: *E. EKHTIARI BIDHENDI[1], P. M. ANDERSEN[1], S. L. MARKLUND[2], T. BRÄNNSTRÖM[2];
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Abstract: ***Background:*** In the last decade, evolving evidence suggest the presence of propagating prion-like species in severaltypes of neurodegenerative diseases associated with misfolding of host-encoded non-mutated proteins. Among theseare AD, PD, the tauopathies, HD and ALS. A hallmark of prions is the presence of different conformational aggregatestrains with different biological activities. Another characteristic of prions is that subsequent prion passage within thesame host can lead to a shorter incubation period. In ALS, using binary epitope mapping (BEM) we have identifiedtwo structurally different strains of mutant hSOD1 aggregates (named A and B) in the CNS of Tg mice modelsexpressing full-length hSOD1 variants. When seeded into spinal cords of adult hSOD1^{G85R} Tg mice, these strainstransmit exponentially propagating templated hSOD1 aggregation selectively in the motor system with concomitantdevelopment of muscle wasting and paresis. ***Objective:*** To further investigate prion-like properties of hSOD1 strainsin ALS, and explore the spreading characteristic of the disease *in vivo*. ***Methods:*** Stereotaxic inoculation of hSOD1aggregate strains into spinal cords of pre-symptomatic Tg-hSOD1^{D90A} mice were performed. This mutation isessentially wt-SOD1 like. Normally, Tg-hSOD^{D90A} only develop motor neuron disease phenotype when the transgeneis homozygous. Mice hemizygous for the hSOD1^{D90A} transgene insertion do not spontaneously develop ALSpathology and have a normal murine lifespan (>700 days). First & second passage studies were performed toinvestigate further prion-like properties of hSOD1. ***Results:*** Inoculations of either strain A or B seeds into the lumbarspinal cord of 100-day-old hemizygous hSOD1^{D90A} mice induced progressive hSOD1 aggregations and prematurefatal ALS-like disease after ≈250 and ≈350 days, respectively. BEM analysis of aggregates in the terminal stage leadto a surprise discovery: Inoculation of strain A into hemizygous hSOD1^{D90A} mice gave rise to a new strain named C,which has the C-terminal end apparently recruited to the aggregate core.

Second passage inoculations were thenperformed in hemizygous hSOD1^{D90A} mice, using spinal cord homogenates with strain C aggregates as seeds. Thenovel prion strain was much more efficient, and transmitted progressive hSOD1 aggregation and ALS-like diseasewhich was fatal already 100 days after inoculation. The resulting conformational changes are under investigation.***Conclusion:*** We provide further evidence of the similarities between hSOD1 and the prion protein. Our data suggestthat mutations in SOD1 are inducing aggregation and ALS pathology via a prion mechanism.

Disclosures: E. Ekhtiari Bidhendi: None. **P.M. Andersen:** None. **S.L. Marklund:** None. **T. Brännström:** None.

Poster

P285. Animal Models I

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Program #/Poster #: P285.08

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: Leon Levy Neuroscience Fellowship

Title: Linking molecular abnormalities to behavioral deficits using a zebrafish model for tauopathies

Authors: *Y. ZHU[1], P. LEARY[1], Q. BAI[2], E. A. BURTON[2,3], D. SCHOPPIK[1];
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Abstract: Tauopathies are neurodegenerative diseases characterized pathologically by accumulation of abnormal Tau in the brain. However, it remains unclear how these molecular and cellular dysfunctions lead to behavioral deficits, especially during the early stages of pathogenesis. To dissect disease mechanisms across multiple biological scales, we generated a zebrafish model of progressive supranuclear palsy (PSP), a primary tauopathy causing unexpected falls in patients early in disease progression, by expressing human 0N/4R-Tau in the evolutionarily conserved vestibulospinal (VS) nucleus. Human Tau-expressing zebrafish exhibit impaired balance control during free-swimming while maintaining normal locomotor ability compared to their siblings. Functional imaging of the VS nucleus shows decreased calcium signals in Tau-expressing neurons in response to tilt stimulus. This altered neuronal activity correlates with Tau phosphorylation in VS neurons. Interestingly, we also observed ectopic accumulation of acidic organelles in the cell bodies of Tau-positive neurons, suggesting abnormal lysosomal function. Taken together, our zebrafish PSP model allows us to understand

molecular and cellular mechanisms of balance deficits in tauopathies and can be a powerful system for preclinical drug screening and evaluation of potential therapeutic targets.

Disclosures: Y. Zhu: None. **P. Leary:** None. **Q. Bai:** None. **E.A. Burton:** None. **D. Schoppik:** None.

Poster

P285. Animal Models I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P285.09

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Support: R41 AG060836

Title: Protective Effects of a Small Molecule Inhibitor, DDQ Against phosphorylated tau in Alzheimer's Disease

Authors: *M. GEORGE, M. VIJAYAN, L. BUNQUIN, C. BOSE, H. REDDY;
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Abstract: Abstract: The purpose of our study is to determine the protective effects of the newly discovered molecule DDQ (diethyl (3,4-dihydroxyphenethylamino) (quinolin-4-yl) methylphosphonate) against mutant Tau and phosphorylated tau (p-Tau) in Alzheimer's disease (AD). To achieve our objective, we used a well-characterized Tau (P301L) transgenic mouse model. **Methods:** We administered DDQ, a 20 mg/kg body weight (previously determined in our laboratory) intra-peritoneally 3-times per week for 2 months, starting at the beginning of the 12^{th} month until the end of the 14^{th} month. Further, using biochemical and molecular methods, we measured the levels of DDQ in the blood, skeletal muscle, and brain. Using q-RT-PCR, immunoblotting, and immunofluorescence, we studied mRNA and protein levels of mitochondrial dynamics, mitochondrial biogenesis, synaptic, and sirtuins, in DDQ-treated Tau mice. **Results:** Our extensive pharmacodynamics analysis revealed high peak levels of DDQ in the skeletal muscle, followed by serum and brain. Interestingly, mitochondrial dynamics, mitochondrial biogenesis, synaptic, sirtuin mRNA, and protein levels were upregulated in DDQ-treated Tau mice relative to untreated Tau mice. Our immunostaining data concurred with mRNA (q-RT-PCR) and protein (immunoblotting) data. **Conclusions:** Together with our previous study observations, current study findings strongly suggest that DDQ has anti-aging and anti-p-Tau effects and a promising molecule to reduce age-and p-Tau-induced toxicities in AD.

Disclosures: M. George: None. **M. Vijayan:** None. **L. Bunquin:** None. **C. Bose:** None. **H. Reddy:** None.

Poster

P285. Animal Models I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P285.10

Topic: C.05. Tauopathies, Tau-dementias, and Prion Diseases

Title: The ketone body beta-hydroxybutyrate ameliorates molecular and behavioral pathological markers in a Drosophila model of glial tauopathy.

Authors: *C. D. DAHMANI[1], T. O'TOOLE[1,2], J. O'CONNELL[3], D. C. LEE[2,1], K. J. COLODNER[3], G. R. TANNER[1,4];
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Abstract: Tauopathies are a class of neurodegenerative diseases characterized by abnormal intracellular aggregations of the microtubule-stabilizing protein tau. Symptomology of tauopathies includes impaired cognition—including difficulties with learning and memory—caused by progressive neuronal and synaptic degeneration. Tau pathology is often characterized by an accumulation of hyperphosphorylated tau protein resulting in the formation of neurotoxic tau aggregates, also known as neurofibrillary (NFT) and gliofibrillary (GFT) tangles. These aggregates may hinder neural glycolytic metabolism and result in degradation of the myelin sheath and other glial components. Ketone bodies (KBs), metabolites of the putatively-neuroprotective high-fat and low-carbohydrate ketogenic diet (KD), bypass cytoplasmic glycolysis and enter directly into mitochondrial metabolism. The KD and KBs serve to elevate cellular ATP/ADP ratio and thus may rescue neural energetics. We have obtained evidence strongly suggesting that KBs may exhibit neuroprotective effects in a *Drosophila melanogaster* model of traumatic brain injury (TBI); repeated TBI may lead to the progressive neurodegenerative condition termed Chronic Traumatic Encephalopathy (CTE), which is characterized in part by intracellular aggregation of tau protein in neural cells. We employed a *Drosophila* model of tauopathy that allows for spatial restriction of tau protein overexpression to glial cells and for temporal restriction of tau overexpression to adulthood, so as to avoid developmental effects of neural tau protein overexpression. We directly applied a racemic mixture of the KD metabolite beta-hydroxybutyrate (β-HB)—the major circulating ketone body (KB)—to a standard high-carbohydrate *Drosophila* diet to determine whether dietary KB supplementation can ameliorate tauopathy-associated learning deficits. We found that β-HB markedly improves tauopathy-induced learning deficits in mixed-sex groups of adult *Drosophila* and reduces levels of phosphorylated tau in the brains of male flies subjected to induced glial tauopathy.

Disclosures: C.D. Dahmani: None. **T. O'Toole:** None. **J. O'Connell:** None. **D.C. Lee:** None. **K.J. Colodner:** None. **G.R. Tanner:** None.

Poster

P392. Pain Models: Behavior I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P392.01

Topic: D.03. Somatosensation – Pain

Support: NIH Grant AR061371
NIH Grant AR073187

Title: Inhibition of P2X7/NLRP3/Caspase-1 pathway in chronic muscle pain

Authors: *K. HAYASHI, J. B. LESNAK, L. RASMUSSEN, K. A. SLUKA;
Univ. of Iowa, Iowa, IA

Abstract: Activity-induced muscle pain is a significant clinical problem that contributes to disability and reduced function. We developed an animal model of activity-induced muscle pain that is dependent on local macrophage activation and release of interleukin-1β (IL-1β). Production of IL-1β in macrophages occurs via activation of purinergic receptor-7 (P2X7), NOD-like receptor protein 3 (NLRP3), and Caspase-1. We hypothesized that pharmacological blockade of P2X7, NLRP3, Caspase-1 before induction of the pain model will prevent development of muscle pain through release of inflammatory cytokines. Muscle pain was induced by 2, 20μl pH 5.0 injections, 5 days apart, in the gastrocnemius muscle combined with fatiguing muscle contractions in male and female C57BL6/J mice. To assess hyperalgesia, muscle withdrawal thresholds (MWT) of the gastrocnemius muscle were measured bilaterally before and 1, 3 and 7 days after induction of the model in the following groups injected intramuscularly: P2X7 antagonist A740003 (0.12-1.2μM, 20μl), NLRP3 inhibitor MCC950 (2-20nM, 20μl), Caspase-1 inhibitor Z-WEHD-FMK (1.2-12μM, 20μl), IL-1β immunizing antibody. Release of cytokines and mRNA expression of P2X7, NLRP3 and Caspase-1 were assessed from muscle and resident macrophages using multiplex and qPCR, respectively. The decrease in MWT in male, but not female, mice was prevent by intramuscular blockade of P2X7 ($p<0.01$; 1,507 ± 22mN with 1.2μM vs. 1,151 ± 31mN vehicle), NLRP3 ($p<0.01$; 1,467 ± 17mN with 20.0nM vs. 1,197 ± 18mN vehicle), and Caspase-1 ($p<0.01$; 1,448 ± 54mN with 12.0μM vs. 1215 ± 37mN vehicle). Blockade of IL-1β before induction of the model prevented hyperalgesia in male and female mice; while blockade 1 day after induction of the model blocked hyperalgesia in males only. In parallel, after induction of the model, there was an increase in interleukin-6 (M, 9.9pg/ml vs. 7.2pg/ml; F, 9.2pg/ml vs. 8.6pg/ml), tumor necrosis factor alpha (M, 1.7pg/ml vs. 0.8pg/ml; F, 4.5pg/ml vs. 1.1pg/ml), and interferon-gamma (M, 1.0pg/ml vs. 0.6pg/ml; F, 2.5pg/ml vs. 1.0pg/ml) protein, and P2X7 (M, 1.7-; F, 4.3-fold), NLRP3 (M, 1.9-; F, 4.0-fold), Caspase-1 (M, 3.5-; F, 3.2-fold), and IL-1β (M, 33.4-; F, 19.2-fold) mRNA expression in muscle tissue when compared to controls in male and female mice. The current data suggests

activation of P2X7, NLRP3, and Caspase-1 initiates development of muscle pain in male, not in female mice, and is associated with increased release and expression of inflammatory cytokines in male and female mice. Thus, we show sex-dependent mechanisms for development of activity-induced pain that uses a P2X7-NLRP3-Caspase-1 pathway in males to produce hyperalgesia.

Disclosures: K. Hayashi: None. **J.B. Lesnak:** None. **L. Rasmussen:** None. **K.A. Sluka:** None.

Poster

P392. Pain Models: Behavior I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P392.02

Topic: D.03. Somatosensation – Pain

Support: NIH Contract No 75N95019D00026

Title: Characterization of behavioral pain phenotype and pharmacology in the rat monoiodoacetate model of osteoarthritis pain.

Authors: *M. URBAN[1], E. DUGAN[1], K. BUBAN[1], J. HAGEDORN[1], P. WIG[1], S. A. WOLLER[2], S. IYENGAR[2], T. HANANIA[1];
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Abstract: In collaboration with the NIH HEAL Initiative Preclinical Screening Platform for Pain (PSPP), we evaluated a variety of pain behaviors in the rat monoiodoacetate (MIA) model of osteoarthritis in male and female Sprague Dawley rats for a period of 4-6 weeks following MIA injection (0.3 - 3 mg). Effects of reference analgesic compounds were also evaluated following acute and chronic treatment to examine pharmacology. Intraarticular injection of MIA (1, 3 mg) into the hindlimb knee joint produced unilateral hind paw tactile hypersensitivity in male and female rats which was maximal at Week 2. Unilateral knee joint hypersensitivity to pressure and pinch stimuli was observed in female, but not male, rats at Week 2. Weight bearing deficits associated with the affected hind limb were modest when measuring static weight bearing, and were more pronounced when measuring dynamic weight bearing in male and female rats, with maximal effects observed at Week 1. Changes in gait were also observed in male and female rats following MIA injection, with significant differences observed in hind paw speed, rhythmicity, and paw position at Weeks 1 and 2. The optimal dose of MIA to examine pharmacological effects in this model was determined to be 1 mg, based on lower variability in weight bearing responses. Acute treatment with morphine (3 mg/kg s.c.) reduced hind paw tactile hypersensitivity and weight bearing deficits in male and female rats, whereas acute treatment with ketoprofen (6 mg/kg s.c.) or duloxetine (60 mg/kg p.o.) was partially effective or ineffective. In contrast, chronic treatment with ketoprofen or duloxetine (4 days b.i.d.)

significantly reduced tactile hypersensitivity and weight bearing deficits. The results from this study demonstrate that a variety of pain behaviors associated with knee joint osteoarthritis can be measured using the rat MIA model. An acute and chronic treatment paradigm may be used to identify and differentiate novel mechanisms for the treatment of pain associated with osteoarthritis.

Disclosures: M. Urban: None. **E. Dugan:** None. **K. Buban:** None. **J. Hagedorn:** None. **P. Wig:** None. **S.A. Woller:** None. **S. Iyengar:** None. **T. Hanania:** None.

Poster

P392. Pain Models: Behavior I

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Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P392.03

Topic: D.03. Somatosensation – Pain

Support: Jordan Univ.Sci.Tech Deanship of research

Title: Evaluation of sucrose induced antinociception on long term effects of neonatal noxious stimulation in rats

Authors: *K. NUSEIR, K. ALZOUBI, *A. ALTARIFI;
Jordan Univ. of Sci. and Technol., Irbid, Jordan

Abstract: Pain during infancy have dire consequence both short and long term. Procedural pain is induced as tests and treatments are needed when premature and mature babies are admitted to NICU. Sadly, pain management is either not available or not adequate. Sweet solutions have been shown to decrease some of this pain, as well as prevent negative sequalae of neonatal pain. Mechanism of sweet solution induced pain relief is largely unknown yet. To explore the effects of sucrose solution on induced repetitive nociceptive stimulation and its long-term effects, this work is proposed. Briefly, a small needle will be inserted and removed quickly in rat pups' paws during their first two weeks of life. Sucrose or saline will be applied to the tongues of these pups just before noxious stimulation. At adulthood, a battery of behavioral tests will be run in sequences in order to explore deficiencies and inadequacies that result from pain. By employing both male and female rats to examine sex differences if any. Biochemical markers will also be tested to add to our knowledge of the mechanisms of sweet induced analgesia.

Disclosures: K. Nuseir: None. **K. Alzoubi:** None. **A. Altarifi:** None.

Poster

P392. Pain Models: Behavior I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P392.04

Topic: D.03. Somatosensation – Pain

Support: NIH Grant R01 AA025024

Title: Contribution of main olfactory system to mechanical hyperalgesia in mice exposed to bedding from CFA-treated conspecifics

Authors: *Y. ZHANG, A. E. RYABININ;
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Abstract: Rodents can develop nociceptive hypersensitivity after interacting with familiar conspecifics in painful conditions. Our laboratory has previously shown that mice repeatedly exposed to, but not physically contacting, the bedding from conspecifics in persistent painful conditions also developed long-lasting nociceptive hypersensitivity. This finding provided evidence that the nociceptive behavior of mice could be socially modulated without direct interaction, potentially via olfactory system. To further understand this phenomenon, the current study aimed to investigate whether a brief exposure to the bedding from conspecifics in pain would modulate the nociception in mice, and whether the olfactory system was involved in such modulation. Female C57BL/6J mice were singly housed in separate cages with only wire lids but no filter covers. After habituation, they were randomly selected as test mice and stimulus mice. Test mice were further assigned to four treatment groups that were exposed to one of the four different stimuli: a familiar stimulus mouse treated with Complete Freund's Adjuvant (CFA), a familiar stimulus mouse treated with saline, and the bedding from a CFA- or saline-treated stimulus mouse. The von Frey mechanical thresholds of the test mice were measured before and at 1, 2, 4 and 24hr after a one-hour exposure to the stimuli. We found that exposure to the bedding from CFA-treated stimulus mice induced an acute mechanical hypersensitivity in the test mice from 1hr to 4hr after the exposure, similar to the effect of exposure to a CFA-treated stimulus mouse itself. In contrast, in female mice with dichlobenil-induced lesion in the olfactory epithelium, the bedding from CFA-treated stimulus mice did not produce mechanical hypersensitivity. This result indicates that the nociception modulatory effect of the bedding exposure requires the functional olfactory epithelium in test mice. Taken together, this study demonstrates that a single no-contact exposure to the bedding of conspecifics in pain leads to transient nociceptive hypersensitivity in mice that is likely mediated via the main olfactory system.

Disclosures: Y. Zhang: None. **A.E. Ryabinin:** None.

Poster

P392. Pain Models: Behavior I

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Topic: D.03. Somatosensation – Pain

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Title: Automated detection of mouse pain behavioral readouts by alternating bottom-up pose and paw contact measurements

Authors: *Z. ZHANG[1,2,3], M. KOTODA[4,3], B. TURNES[4,3], D. P. ROBERSON[3,2], B. BOIVIN[4,3], C. J. WOOLF[5,3];
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Abstract: The lack of robust quantifiable behavioral assessments of pain in preclinical models, especially of ongoing pain, has been a major limitation for both pain neurobiology research and the development of novel analgesics. Conventional methods typically require extensive animal-human observer interaction and are often followed by manual, thus subjective, scorings, which are susceptible to bias and variability, and are labor-intensive and time-consuming. With recent advancements in both high-speed videography and machine-learning-powered video processing and behavioral analysis, conventional methods have been automated, modified, and new methods have been developed. However, behavioral data analysis with advanced machine learning remains a technical niche, with limited accessibility to the more general pain research community and for large-scale pre-clinical novel analgesic screening. Here we demonstrate that, by leveraging modern machine vision and machine learning tools, quantifiable features of the spontaneous behavior, recorded in a dark and observer-free environment from a bottom-up view, with simultaneous surface contact measurements, can be directly used to detect and infer both the internal state of pain and analgesia in an animal, in an objective manner. We further optimize and automate a robust pain behavioral assay, with a complete hardware and software solution that is user-friendly, for exploring the biology of pain, one readily scalable for pre-clinical drug efficacy assessment.

Disclosures: Z. Zhang: None. **M. Kotoda:** None. **B. Turnes:** None. **D.P. Roberson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent holder. **B. Boivin:** None. **C.J. Woolf:** E.

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

Poster

P392. Pain Models: Behavior I

Location: Virtual Only

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Program #/Poster #: P392.06

Topic: D.03. Somatosensation – Pain

Support: NIH Grant R01 DE029074

Title: Sex differences in orofacial sensitivity, visceral sensitivity, and brain activity in an animal model of stress-induced pain hypersensitivity

Authors: L. HERNANDEZ[1], J. DA SILVA[1], S. HANSON[1], A. J. SCOTT[2], R. K. ERNST[2], O. K. MELEMEDJIAN[1], D. A. SEMINOWICZ[1], *R. J. TRAUB[1];
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Abstract: Temporomandibular Disorder and irritable bowel syndrome are two of ten Chronic Overlapping Pain Conditions, conditions that tend to co-occur and are predominant in women. However, mechanisms underlying the comorbidity of chronic pain originating in distally unrelated tissue types are unknown. We hypothesize changes in brain function initiated by pre-existing pain combined with stress contributes to development of *de novo* pain in susceptible individuals. Using an animal model (orofacial pain plus stress) that induces *de novo* Comorbid visceral Pain Hypersensitivity (CPH) in rats, we reported more robust and longer duration CPH in female rats compared to males (Da Silva, IASP 2021 abstract). fMRI revealed enhanced activity in the insula, parabrachial nucleus, and gigantocellular reticular nucleus in naïve females. Activity in the anterior cingulate cortex, nucleus accumbens, thalamus, hippocampus, and substantia nigra was increased over time in females. In contrast, males were hypersensitive to distention at one week, with hypersensitivity resolved by four weeks. Brain activity in males at seven weeks was similar to the baseline state, which showed increased activation of the thalamus and periaqueductal gray, as compared to one week. In the present study, the effects of stress alone (Stress-Induced Hypersensitivity, SIH) were examined. The relative percentage of male (9/12) and females (8/14) rats susceptible to stress (increased visceral pain one week post stress) was comparable. The stress resilient rats were excluded from further analysis of visceral pain. Preliminary data from both males and females showed SIH four weeks post stress, resolving by seven weeks in females (ANOVA, females p=.0250); data collection ongoing in males. Additional preliminary data show stress alone increased orofacial mechanosensitivity in females (p=.0210, n=5), but not males (p=0.4, n=5). In the female cohort, the increased orofacial pain was only apparent in the susceptible females and not the resilient females. Analysis of functional connectivity using fMRI is underway. Ultimately, changes in functional connectivity

in SIH rats will be compared to CPH rats. This study supports our previous work comparing SIH and CPH conditions showing many similarities, but also differences, especially duration of pain hypersensitivity that is correlated with changes in brain function contributing to sex differences in chronic pain.

Disclosures: L. Hernandez: None. **J. Da Silva:** None. **S. Hanson:** None. **A.J. Scott:** None. **R.K. Ernst:** None. **O.K. Melemedjian:** None. **D.A. Seminowicz:** None. **R.J. Traub:** None.

Poster

P392. Pain Models: Behavior I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P392.07

Topic: D.03. Somatosensation – Pain

Title: 10kHz spinal cord stimulation alleviates mechanical hypersensitivity in a rat model of painful diabetic neuropathy

Authors: *D. WANG, K. LEE, Z. KAGAN, D. LEE, K. BRADLEY;
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Abstract: Painful diabetic neuropathy (PDN) is a serious complication of diabetes and occurs in approximately 50% of older patients with diabetes mellitus. In a recent randomized controlled clinical trial, 10kHz spinal cord stimulation (10kHz SCS) was shown to be a promising therapy for PDN, in both pain reduction and possible neurological improvements (Petersen et al 2021). In this study, we aimed to observe the behavioral and neural effects of 10kHz SCS on PDN using streptozotocin (STZ)-induced diabetic rats. We established four testing groups: naïve controls, STZ controls (STZ injection only), STZ+Sham (STZ injection with implanted-but-unstimulated epidural spinal electrode), and STZ+10kHz SCS (STZ injection with implanted-and-stimulated epidural spinal electrode). A single effective dose (60 mg/kg) of the cytotoxic agent STZ caused the rats to become hyperglycemic (>270 mg/dl) within 72 hours and lasted for several weeks. These STZ-injected rats also showed a significant and continuous reduction in body weight in comparing to naïve control rats. In behavioral assessments, we observed that 7 days of continuous (24h/day) low-intensity (30% of motor threshold) 10kHz SCS appeared to restore von Frey paw withdrawal thresholds to approximately 96% of naïve controls, showing statistically significant improvement from the Pre-SCS baseline after 7 days of 10kHz SCS, and versus STZ control or STZ+Sham animals ($p<0.05$). In addition, we performed electrophysiologic testing just prior to termination in all groups. We observed normalization of the receptive field area of wide dynamic range dorsal horn neurons with 10kHz SCS: STZ control and STZ+Sham rats demonstrated significantly expanded receptive fields compared to naïve, while STZ+10kHz SCS rats trended towards reduction of receptive field size towards

naïve controls.
Our results suggest that 10kHz SCS resulted in behavioral outcomes reflective of pain reduction, as well as normalization trends of central neuron processing in PDN animal model. These findings provide supporting evidence for recent randomized clinical trial data of 10kHz SCS and underscore the conclusion that 10kHz SCS can effectively treat patients with refractory PDN.

Disclosures: D. Wang: A. Employment/Salary (full or part-time):; Nevro Corp. **K. Lee:** A. Employment/Salary (full or part-time):; Nevro Corp. **Z. Kagan:** A. Employment/Salary (full or part-time):; Nevro Corp. **D. Lee:** A. Employment/Salary (full or part-time):; Nevro Corp. **K. Bradley:** A. Employment/Salary (full or part-time):; Nevro Corp.

Poster

P392. Pain Models: Behavior I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P392.08

Topic: D.03. Somatosensation – Pain

Support: Duke University Anesthesiology Research funds
Duke Microbiome Center Grant
the Duke Cancer Institute P30 Cancer Center Support Grant

Title: Behavioral characterization of humanized mice: a platform to understand chronic graft-versus host disease-associated pain?

Authors: E. HEZARKHANI[1], A. S. ANDRIESSEN[1], J. ZHAO[1], S. BANG[1], B. A. MOCK[5], J. W. MAYS[6], R.-R. JI[1,2,3], J. T. NGUYEN[6,5], C. R. DONNELLY[1,4];
[1]Ctr. for Translational Pain Medicine, Dept. of Anesthesiol., [2]Dept. of Cell Biol., [3]Dept. of Neurobio., [4]Duke Cancer Inst., Duke Univ. Med. Ctr., Durham, NC; [5]Lab. of Cancer Biol. and Genetics, Ctr. for Cancer Research,, NIH, Bethesda, MD; [6]Oral Immunobiology Unit Natl. Inst. of Dent. and Craniofacial Res., Natl. Inst. of Hlth., Bethesda, MD

Abstract: Humanized mice - immunodeficient mice with stably engrafted and functional human cells - have emerged as a leading preclinical platform to study a wide variety of human diseases. Since its development in 2014, the triply immunodeficient “NCG” mouse model, which bears mutations in *Sirpa, Prkdc* and *Il2rg* loci, has emerged as a leading platform to produce humanized mice, enabling the engraftment of human peripheral blood mononuclear cells (huPBMCs) or human umbilical cord derived CD34+ hematopoietic stem cells (huCD34-HSCs). In this study, we characterized the behavioral phenotypes in huCD34-NCG mice 12-15-weeks post-engraftment. Interestingly, although huCD34-NCG mice appear to be overly normal in size and gross health assessment measures compared to non-engrafted NCG mice, we found that huCD34-NCG mice exhibited a variety of behavioral features suggestive of ongoing chronic

pain, including: (1) mechanical hypersensitivity, as determined by von Frey testing and Randall-Selitto testing; (2) thermal hyperalgesia, as determined by hot plate testing; (3) cold allodynia, as determined by plantar acetone testing and cold plate testing; and (4) orofacial allodynia, as determined by consumption of capsaicin-laden drinking water. Neither non-engrafted NCG nor huCD34-NCG mice exhibited signatures of spontaneous pain at baseline. Notably, huCD34-NCG mice and un-engrafted NCG mice appear similar in behavioral tests assessing cognitive function, anxiety, and depression. Given the abundance of literature demonstrating that various humanized mouse models develop graft-versus-host disease after xenogeneic transplants, we posit that huCD34-NCG mice may be a useful platform to study the neurological sequalae associated with chronic graft-versus host disease (GVHD), including chronic pain and its associated comorbidities. Ongoing work is focused on analyzing signatures of neuroinflammation in the peripheral and central nervous systems of huCD34-NCG mice relative to non-engrafted controls. This model may yield new insights into the pathogenesis of pain in GVHD, prompting new therapeutic targets.

Disclosures: E. Hezarkhani: None. **A.S. Andriessen:** None. **J. Zhao:** None. **S. Bang:** None. **B.A. Mock:** None. **J.W. Mays:** None. **R. Ji:** None. **J.T. Nguyen:** None. **C.R. Donnelly:** None.

Poster

P392. Pain Models: Behavior I

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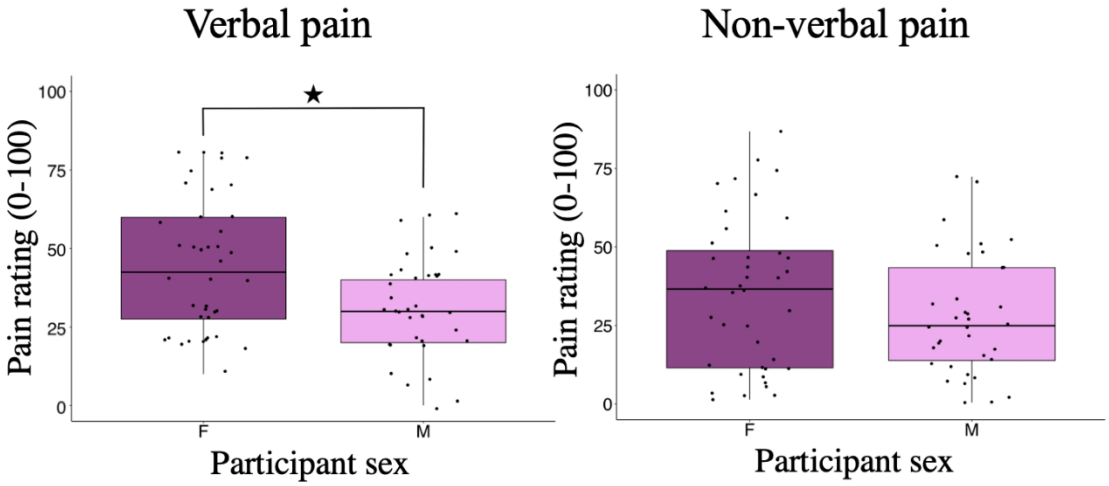
Topic: D.03. Somatosensation – Pain

Title: Examiner gender effect on pain responses

Authors: *N. G. BAILEY[1], J. MCDOUGALL[2], R. BANGA[3], L. D. LINDE[3], J. L. K. KRAMER[1];
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Abstract: Introduction: Pain is reportedly influenced by the sex and gender of the participant and the examiner. This has been chiefly examined using static measurements (e.g., pain thresholds), which are susceptible to bias. This study investigated the influence of examiner and participant's gender and sex on tonic heat responses.**Methods**: 38 participants (18 males, 20 females) attended two identical testing sessions. Participants were randomly assigned to a male or female examiner on day 1, and the opposite examiner on day 2. Rating- and temperature-based methods of evaluating tonic heat were conducted. The rating-based method required participants to rate stimulus intensity using a computerized visual analog scale (CoVAS). Participant controlled temperature (PCT) was used as a temperature-based method. During PCT,

participants changed the temperature of the probe in real-time to reflect shifts in their heat perception, with the goal of maintaining their initial awareness. Participants verbally rated their pain at the start and end of both tests.**Results**: Female participants verbally report higher pain ratings to tonic heat compared to their male counterparts (main effect: F=5.77, p=0.02). Neither CoVAS nor PCT were significantly different based on the sex of the participant (main effects: CoVAS, F=1.20, p=0.28; PCT, F=1.02, p=0.32). Female participants had significantly greater verbal pain ratings during CoVAS when tested by the female examiner (F=5.61, p=0.02). Similarly, females reported greater CoVAS non-verbal pain ratings when tested by the female examiner, albeit non-significant (F=2.70, p=0.11). PCT outcomes were not modulated by the examiner (interaction effect: F=0.45, p=0.51).**Conclusions**: In line with previous studies, female participants tended to verbally report higher pain ratings to tonic heat stimulation compared to males. Non-verbal methods of reporting pain may be less susceptible to gender differences. Temperature-based non-verbal approaches may provide less biased estimates of neurophysiological processes underlying pain.



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Poster

P392. Pain Models: Behavior I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P392.10

Topic: D.03. Somatosensation – Pain

Support: TWU Center for Student Research (CSR)
Department of Biology

Title: Euphorbia bicolor latex extract reduces mechanical allodynia in a rat model of thermal injury

Authors: *T. P. OLAOLUWA, C. MAIER, D. L. AVERITT;
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Abstract: Burn injury is associated with oxidative stress and prolonged inflammation which can lead to chronic pain. Current treatment for pain associated with burns include oral and intravenous analgesics such as non-steroidal anti-inflammatory drugs, opioids, and gabapentinoids which have all been linked to undesired negative side effects. As burns represent a particularly challenging type of injury to treat, novel strategies that mitigate pain related to burns are needed. Our previous studies indicate that the extract of *Euphorbia bicolor,* a plant native to the southern United States, may provide a novel analgesic as it demonstrates anti-inflammatory, antinociceptive, and antioxidant properties. Phytochemicals in the *E. bicolor* extract can directly reduce nociceptor activity, reduce proinflammatory cytokines/chemokines, and reduce reactive oxygen species (ROS). Further, *E. bicolor* latex extract induces long-lasting, non-opioid peripheral analgesia in two rat models of inflammatory pain. As burn pain involve sensory neuron damage, oxidative stress, and inflammation, *E. bicolor* extract may effectively target each of these mechanisms providing a multimodal therapeutic for treating burn pain. We hypothesized that *E. bicolor* latex extract will reduce mechanical allodynia in a rat model of pain associated with full thickness thermal injury. Two concentrations of *E. bicolor* ethanol latex extract, 100 μg/mL and 500 μg/mL, in 0.9% sterile saline, were used. Adult male Sprague-Dawley rats (200-300 g) received a full thickness thermal injury to the plantar surface of the right hind paw by applying a soldering iron tip at 100°C for 30 seconds. Mechanical allodynia was then recorded 24, 48, and 72 hrs post-injury to confirm development of pain behaviors. Rats then received one intraplantar injection of either vehicle or *E. bicolor* latex extract immediately adjacent to the thermal injury and mechanical allodynia was re-examined at 1, 3, 6, 24, 48, and 72 hrs post-injection then once weekly until the thermal injury resolved. Here we report that mechanical allodynia develops at 24 hrs following thermal injury, similar to our previous reports characterizing this model. Peripheral analgesia was then detected within 24 hrs of injection of the 500 μg/mL dose of *E. bicolor* extract. These data confirm that *E. bicolor* latex extract contains phytochemicals that evoke potent analgesia in a rat model of burn pain. Our current studies are focused on determining the individual phytochemicals that contribute to the anti-inflammatory, antioxidant, and antinociceptive properties of *E. bicolor* that account for the observed analgesia to develop into novel non-opioid pain therapeutics.

Disclosures: T.P. Olaoluwa: None. **C. Maier:** None. **D.L. Averitt:** None.

Poster

P406. Opioid Treatment

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Program #/Poster #: P406.01

Topic: D.03. Somatosensation – Pain

Support: NIH 1RF1NS113840-01

Title: Opioid-sparing effects of the Nrf2 activator diroximel fumarate in mice

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Abstract: Opioids are widely used to treat acute and chronic pain. However, their long term administration can result in tolerance and paradoxical opioid-induced hyperalgesia (OIH). Underlying mechanisms include induction of reactive oxygen and nitrogen species (ROS/RNS) in the periaqueductal gray, hippocampus and prefrontal cortex regions of the brain and the dorsal horn of the spinal cord that disrupts glutamate homeostasis, contributing to dysregulation of opioid analgesia. We hypothesized that activating the antioxidant transcription factor Nrf2 would alleviate morphine tolerance and OIH. Diroximel fumarate (DRF), an FDA approved drug, was used to activate Nrf2. For the OIH experiment, naïve mice were implanted with subcutaneous osmotic minipumps to infuse morphine dose for 7 days (10 mg/kg/day). Oral DRF (100 mg/kg/day) or vehicle was administered the day before minipump implantation, and then daily for the 7-day morphine infusion. For the tolerance experiment, naïve mice were implanted with subcutaneous osmotic minipumps to infuse morphine for 5 days (10 mg/kg/day). Oral DRF (100 mg/kg/day) or vehicle were administered the day before minipump implantation, and then daily for the 5-day morphine infusion. On day 5, cumulative doses of morphine (1.8, 3.2, 5.6, 10.0 and 18.0 mg/kg) were injected after every 20 min. Withdrawal thresholds to heat and mechanical stimuli were assessed using hot plate tests and Von Freys, respectively. Male and female mice treated with morphine alone had decreased withdrawal thresholds to heat and mechanical stimuli by day 7, indicating development of OIH. However, DRF co-treatment blunted development of OIH. Mice treated with DRF alone did not show any change in withdrawal thresholds. Effects of DRF on morphine tolerance will be presented. Expression of antioxidant genes/proteins in the spinal cord and brain will also be presented and Nrf2 positive cells identified. Future studies will investigate the effects of DRF on other adverse effects of opioids, including abuse liability, respiratory depression, and withdrawal. These promising results suggest that DRF has opioid-sparing properties.

Disclosures: M.S.U. Rasheed: None. **F.R. Cherry:** None. **P.M. Grace:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Biogen.

Poster

P406. Opioid Treatment

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Topic: D.03. Somatosensation – Pain

Support: NIH NIDA grant DA042888
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Rutgers Brain Health Institute
Rutgers New Jersey Medical School

Title: Truncating intracellular C-termini encoded by exon 7 of mu opioid receptor gene, Oprm1, in C57BL/6J mice attenuates fentanyl and methadone tolerance and reward

Authors: *Y. PAN[1,2], J. XU[1], T. ZHANG[2], V. P. LE ROUZIC[2], S. LIU[1], W. KANG[1], A. ABRIMIAN[1], A. MALIK[1], T. KRAFT[1], R. CHIEN[1];
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Abstract: The single-copy gene (*OPRM1*) encoding the mu opioid receptor (MOR) undergoes extensive alternative pre-mRNA splicing, generating multiple splice variants or isoforms conserved from rodents to humans. One splice variant type includes full-length, seven-transmembrane (7TM) carboxyl (C)-terminal variants that are identical except for sequences located at the tip of the intracellular C-terminal tail. The functional relevance of these 7TM C-terminal variants has been demonstrated using *in vitro* cell models showing differences in mu agonist-induced G protein coupling, phosphorylation, internalization, and postendocytic sorting and by differential region- and cell-specific expression. The *in vivo* function of the C-terminal tails has been investigated in C-terminal truncation mouse models. In particular, truncating exon 7 (E7) of the C-terminus in C57BL/6J mice (mE7M-B6) diminishes morphine tolerance and reward without altering physical dependence. The current study further investigates the effect of E7-truncation on fentanyl and methadone actions including tolerance, physical dependence and reward. We observed that in wild-type (WT) B6 mice, both fentanyl (1 mg/kg/day, subcutaneously (s.c.) osmotic pump for 7 days) and methadone (10 mg/kg, s.c. twice a day for 7 days) produced robust tolerance. However, in mE7M-B6 mice both fentanyl and methadone tolerance were significantly attenuated. Fentanyl and methadone reward determined by conditioned place preference were significantly reduced in mE7M-B6 mice when compared to WT B6 mice. These results were like those seen in morphine tolerance and reward in mE7M-B6 mice. On the other hand, fentanyl-induced dependence determined by naloxone- precipitated withdrawal was slightly increased, while methadone dependence was no change. The similarities

and dissimilarities between mE7M-B6 and β-arrestin2 knockout mice in these mu opioid actions suggest that E7-encoded C-terminal sequences are involved in both β-arrestin2-dependent and β-arrestin2-independent signaling pathways. Fentanyl and methadone-stimulated G protein coupling in brain regions of mE7M-B6 mice were examined using [^{35}S]GTPγS autoradiography and [^{35}S]GTPγS binding assay with isolated brain membranes to determine whether region-specific desensitization mechanisms are involved. Fentanyl and methadone-induced β-arrestin2 recruitment in mMOR-1O, a full length E7-associated 7TM variant, was investigated in Be(2)C cells using with a Nanobit-based β-arrestin2 recruitment assay. Together, these data further support the functional relevance of E7-associated splice variants in opioid pharmacology.

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Poster

P406. Opioid Treatment

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Title: Brainstem opioid receptor architecture and neuromodulation of respiratory, sensory, and motor circuits

Authors: *N. F. HUG[1,4], N. MERCER LINDSAY[2,4,3], W. M. MCCALLUM[4], G. SCHERRER[4,5];
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Abstract: Opioids profoundly alter respiratory activity, sensory responses, and motor behaviors, yet the distribution pattern of opioid receptors in brainstem circuits has not been elucidated. Opioid-induced respiratory depression (OIRD) is the leading cause of opioid-related overdose deaths. While there is significant evidence for the direct action of opioids on brainstem

respiratory networks, on which nuclei and cells opioids act to produce OIRD is unclear. Given the differential effects on respiration of selective agonists for the mu, delta, kappa, and nociceptin opioid receptors (MOP, DOP, KOP, and NOP), elucidating brainstem expression of each receptor is critical for comprehensively understanding OIRD. Further, examination of receptor expression throughout the brainstem is important for understanding opioid-mediated neuromodulation of sensory and motor functions. Here we labeled MOP, DOP, KOP, and NOP using immunohistochemistry and knockin reporter mice, and used 3-D reconstructions to reveal each receptor expression across brainstem circuits. We found that the different receptors are occasionally co-expressed in neurons in a few brainstem regions but are most often found in discrete nuclei. Structures where more than one receptor is expressed are typically multimodal or integrative centers e.g., the parabrachial nuclei (PB), Kölliker-Fuse (KF), deep layers of the superior colliculus, or olivary nuclei. Secondary sensory and motor nuclei generally exhibited discrete receptor expression patterns, such as notable DOP labeling in the ventral cochlear nucleus or MOP labeling in the paratrigeminal nucleus and nucleus ambiguous (NA). Segregation of receptor types was even seen within nucleus subregions, i.e., MOP and NOP labeling in the dorsal cortex of the inferior colliculus while DOP is isolated in the central nucleus. We examined all respiratory brainstem nuclei: pattern generators [e.g., preBötzinger complex (preBötC)], sensorimotor integrators (e.g., PB, KF), and motor output (e.g., NA, hypoglossal nucleus). There is structure-specific organization of receptor type, such as dominant MOP expression in the preBötC, contrasting with overlapping expression of KOP and MOP in the KF. Together, the results establish the brainstem opioid receptor architecture, detailing the specific expression pattern of all four receptors, and provide an essential anatomical basis for constructing hypotheses for how the different MOP-expressing neural populations and co-expression with other receptors in respiratory nuclei modulates respiratory function to produce OIRD.

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Poster

P406. Opioid Treatment

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Program #/Poster #: P406.04

Topic: D.03. Somatosensation – Pain

Support: SC INBRE 21-4275

Title: Treatment with a novel Aha1 inhibitor, KU177, improves opioid therapy

Authors: C. HANNA[1], K. A. GABRIEL[3], C. S. CAMPBELL[3], V. M. KLIEBE[5], B. S. J. BLAGG[6], J. M. STREICHER[4], ***W. LEI**[2];

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Abstract: Treatment with a novel Aha1 inhibitor, KU177, improves opioid therapy
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Opioids are commonly used for managing pain across the US. However, the side effects such as tolerance and dependence limit the clinical use of opioid drugs. Our previous studies demonstrated that treatment with Hsp90 inhibitors in the spinal cord enhanced the morphine antinociception and reduced the tolerance in different pain models. However, serious adverse effects, such as liver toxicity, have been demonstrated associated with universal Hsp90 inhibition; Therefore, a better approach targeting the Hsp90 cascade becomes highly demanded. Activator of Hsp90 ATPase Homolog 1(Aha1) is a co-chaperones of Hsp90 and promotes ATPase activity that is necessary for the function of Hsp90. This study aimed to determine the impact of Aha1 inhibitor, KU-177, on morphine antinociception can have an impact on the extent of antinociception effect of opioids. Male and female CD-1 mice were treated with KU-177 via intrathecal injection. The tail-flick was performed to determine the impact of KU-177 on morphine antinociception and tolerance. Further, we chose a murine microglial cell line, BV-2, to investigate the potential mechanisms. Cells were treated with KU177 for measuring the activation of Nrf2 signaling and the expression of downstream genes. We found that treatment with KU-177 enhanced morphine antinociception. KU-177 could also rescue the tolerance induced by continuously being treated with 10 mg/kg morphine for 3 days. Furthermore, KU-177 dose-dependently increased the Nrf2 activation in the BV-2 cells with or without lipopolysaccharides (LPS) stimulation. These findings demonstrate that Aha1 inhibitor could be a potential approach for improving opioid therapy for pain management.

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Poster

P406. Opioid Treatment

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Program #/Poster #: P406.05

Topic: D.03. Somatosensation – Pain

Support: East Carolina University Human Behavior Research Core

Title: Preference for morphine is attenuated in the presence of the dopamine 3 receptor agonist pramipexole

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Abstract: Despite excellent analgesic properties, opioids carry significant risk of misuse, abuse, and addiction, underscoring the need for adjuvants that can maintain effective analgesia while minimizing the dose of opioids needed to achieve the analgesia. Our previous studies demonstrated that combining morphine with the dopamine D3 receptor agonist pramipexole (PPX) enhanced the analgesic effects of morphine even at low doses of morphine and PPX that were ineffective on their own. We hypothesize that this drug combination will provide significant analgesia but not result in the development of drug preference, suggesting a lower addictive potential than the opioid alone.Two-bottle choice was used to test for the development of preference for morphine/PPX combination (10 male and 10 female Long-Evans rats), and for morphine alone (6 male and 6 female rats). Animals had 28 days of continuous access to pairings of: 1) a bottle containing morphine sulfate (0.05mg/ml) + PPX (0.0008mg/ml) dissolved in water with 2% sucrose and a second bottle (control) containing 2% sucrose, or 2) a bottle containing morphine sulfate (0.05mg/ml) dissolved in water with 2% sucrose and a control bottle containing 2% sucrose. Daily fluid intake was measured in ml, and drug consumption expressed as mg of drug/kg for 24 hours. Locomotor testing was performed prior to the start of drug exposure and at 1 and 4 weeks after initiation of exposure to determine effects of drugs on spontaneous behaviors. Preference was determined by comparing mg/kg consumption rates of the 2 solutions each day and as a total consumed over a 21-day exposure period.On days 2-21 of exposure, male rats consumed significantly more morphine solution (mg/kg) than morphine + PPX (mean daily consumption = 1.03 ± 0.16 vs. 0.27 ± 0.06 mg/kg; 95% CI of the mean = 0.4-1.1). Female rats consumed significantly more morphine from the first day of exposure throughout the exposure period (mean daily consumption = 2.74 ± 0.19 vs. 0.64 ± 0.09 mg/kg; 95% CI of the mean = 1.64-2.54). No rats escalated their consumption of morphine/PPX over the exposure period. In contrast, 33% of rats (both male and female) increased consumption from the bottle of morphine alone. Animals exposed to the morphine/PPX combination displayed no changes in locomotor activity, while animals exposed to morphine alone showed increased locomotor activity at 1 week (males) or 4 weeks (females).In conjunction with previous studies showing that PPX enhances the analgesic effects of low dose morphine, these results suggest that the morphine/PPX drug combination represents a potential pain therapy that can be used long-term without producing addictive behaviors.

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Poster

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Topic: D.03. Somatosensation – Pain

Support: NIH R01DA041529

Title: Advanced age and sex modulate multiple mu opioid receptor signaling mechanisms in the rat midbrain periaqueductal gray: Implications for analgesia

Authors: *E. F. FULLERTON[1], M. C. KAROM[1], J. M. STREICHER[2], A. Z. MURPHY[1]; [1]Neurosci. Inst., Georgia State Univ., Atlanta, GA; [2]Pharmacol., Univ. of Arizona, Tucson, AZ

Abstract: Chronic pain is exceedingly prevalent in individuals over 65 years of age but is under-managed in this population, due in part to a dearth of knowledge regarding the suitable dosing of opioids for chronic pain management. We have shown that advanced age and sex alter morphine modulation of persistent inflammatory pain (induced by intraplantar administration of Complete Freund's adjuvant (CFA)), where morphine potency is highest in adult male rats (2mos), with a 2-fold rightward shift in the dose-response curve for aged males (18mos) and females regardless of age. In this study, we tested the hypothesis that advanced age attenuates μ-opioid receptor (MOR) signaling in the ventrolateral periaqueductal gray (vlPAG), a critical region for the modulation of pain. Using a combination of molecular and pharmacological techniques on vlPAG tissue from adult and aged, male and female rats collected 72h following CFA administration, we find that aged males exhibit reduced vlPAG MOR expression (Bmax) compared to adult males, and both aged males and aged females exhibit reduced vlPAG MOR affinity (Kd) compared to adult males. Further, aged males and females, regardless of age, show reduced vlPAG MOR binding potential and reduced G-protein activation efficiency compared to adult males. Together, these results suggest that age- and sex-induced alterations in MOR machinery contribute to reduced opioid potency. The regulation of opioid-induced G-protein signaling was further assessed using RNAscope to analyze mRNA expression of Regulator of G-Protein Signaling (RGS) family members RGS4 and RGS9-2. Our analyses of RGS mRNA revealed increased expression of RGS4 and RGS9-2 in the vlPAG of aged animals compared to adults, indicating that MOR signaling is subjected to greater negative regulation in the aged vlPAG. The observed age-related reductions in vlPAG MOR expression, MOR agonist binding, and opioid-induced G-protein activation, along with the observed increase in vlPAG RGS4 and RGS9-2, as a function of age, have significant implications in pain management in the aged population. We are currently assessing age and sex differences in vlPAG MOR phosphorylation using immunoprecipitation of MOR phosphorylated at serine 375 and assessing opioid-induced cAMP inhibition using TR-FRET cAMP immunoassay to elucidate the mechanisms whereby morphine potency is reduced in aged animals. These novel findings of age-induced attenuation in MOR signaling within the PAG provide potential therapeutic targets to improve pain management in the elderly.

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Poster

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Topic: D.03. Somatosensation – Pain

Support: Arizona Biomedical Research Commission New Investigator Award

Title: Heat shock protein 90 inhibition in spinal cord enhances opioid anti- nociception by suppressing EGFR and AMPK signaling

Authors: *K. GABRIEL[1], J. M. STREICHER[2];
[2]Pharmacol., [1]Univ. of Arizona, Tucson, AZ

Abstract: Opioid misuse is a prevailing problem for chronic pain treatment and is accompanied by side effects such as constipation, tolerance, and addiction. Mu opioid receptor (MOR) signal modulation has been heavily studied in an attempt to optimize opioids and to decrease their side effects. Our lab has demonstrated an important role for the chaperone protein Heat shock protein 90 (Hsp90) in regulating ERK MAPK signaling and pain relief downstream of the MOR. When 17-AAG, an Hsp90 inhibitor, is administered to the spinal cord (intrathecally, IT), there is an augmentation in morphine induced antinociception in mice. This signifies that Hsp90 acts to repress ERK MAPK signaling to reduce antinociception in the spinal cord. This led us to perform quantitative proteomic analysis on the spinal cords of CD-1 mice treated with 0.5 nmol 17-AAG, in order to elucidate the signaling network that activates opioid anti-nociception in the spinal cord. Network analysis of this data revealed that epidermal growth factor receptor (EGFR) was downregulated by 1.4-fold and 5'-AMP-activated protein kinase subunit beta-1 (AMPK) was also downregulated by 1.4-fold. Previous studies have implicated EGFR in promoting opioid receptor internalization and AMPK activation in preventing pathological pain. This result suggested that a reduction in EGFR and AMPK activity could lead to enhanced opioid signaling and pain relief. We tested EGFR agonist EGF (0.01nmol, IT) and found that it decreased 0.5nmol 17-AAG enhanced morphine (3.2 mg/kg, subcutaneous, SC) antinociception in the 52°C tail flick test in both male and female mice. Conversely, when testing the EGFR inhibitor Afatinib (10nmol, IT), there was an enhancement of antinociception in males but not females. Similarly, when using an AMPK agonist AICAR (100nmol, IT) there was a decrease in 17-AAG's enhanced antinociception, but it also decreased morphine's effect in the vehicle group only in male mice. Consequently, when using the AMPK inhibitor dorsomorphin (20 nmol, IT), we were able to see an enhancement of morphine antinociception greater than 17-AAG alone in both males and females. Our data supports our hypothesis that Hsp90 inhibition in spinal cord

enhances opioid pain relief by decreasing EGFR and AMPK signaling that varies between male and female mice. This suggests that these molecules act as negative feedback breaks on opioid anti-nociception in the spinal cord which leads to the enhanced opioid antinociception. This can be reversed when either an EGFR or AMPK agonist is administered, thus elucidating key molecular mechanisms to establish the basic science foundation by which Hsp90 is regulating opioid signaling.

Disclosures: K. Gabriel: None. **J.M. Streicher:** None.

Poster

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Topic: D.03. Somatosensation – Pain

Support: Arizona Biomedical Research Commission New Investigator Award #ADHS18-198875
Institutional funds from the University of Arizona

Title: Inhibition of Heat shock protein 90 in the Spinal Cord Enhances Opioid Anti-Nociception by Disabling an Inhibitory GABA Circuit

Authors: *C. CAMPBELL[1], J. M. STREICHER[2], P. LANGLAIS[3];
[1]Neurosci., Univ. of Arizona Grad. Interdisciplinary Program In Neurosci., Tucson, AZ; [2]Pharmacol., Univ. of Arizona, Tucson, AZ; [3]The Univ. of Arizona, Tucson, AZ

Abstract: Opioid drugs used in the treatment of pain are in desperate need of replacement due to their deleterious side effects. One strategy on offer is developing dose-reductive compounds that, when co-administered with palliatives, can reduce the dose of opioid required for treatment, retaining analgesic efficacy while reducing unwanted side-effects such as addiction, respiratory depression, and death. We previously showed that intrathecal (IT) administration of the heat shock protein 90 (Hsp90) inhibitor 17-AAG potentiated the efficacy of morphine induced antinociception, while reducing or not changing opioid side effects, enabling a dose-reduction strategy. Proteomic analysis of the translational alterations induced by IT 17-AAG administration revealed an upregulation of GABA transporter 2 (GAT-2). To elucidate the role of GAT-2 in mediating the enhancement of morphine antinociception, 17-AAG treated mice were administered a GAT inhibitor, Nipecotic Acid (NA), in the tail flick assay. NA effectively blocked the 17-AAG enhancement of antinociception compared to controls, while not changing responses in Vehicle-treated mice, suggesting that GAT-2 mediates 17-AAG's antinociceptive enhancement. Since GAT-2 takes up GABA into the cell and reduces GABA activity, we hypothesized that an inhibitory GABA circuit is present in the spinal cord which reduces opioid

antinociception and is disabled by Hsp90 inhibition and GAT-2 upregulation. To test this GABA hypothesis, mice were treated with IT GABA-A or GABA-B agonists (muscimol, baclofen) prior to systemic morphine administration. The GABA-A agonist reversed 17-AAG enhanced antinociception much like NA, supporting our hypothesis. Intriguingly, the GABA-B agonist reversed 17-AAG antinociception in males but not females, suggesting a GABAergic sex-specific difference. To further test the inhibitory GABA circuit hypothesis, naïve mice (no 17-AAG) were injected with IT GABA-A or GABA-B antagonists (bicuculline, CGP54626) prior to morphine and tail flick. Males showed enhanced morphine antinociception with either antagonist, supporting the GABA inhibitory circuit hypothesis; however, neither drug had an impact on female mice, again suggesting mechanistic sex differences. These results taken together suggest that IT 17-AAG potentiates morphine-induced antinociception via upregulation of GAT-2, relieving an inhibitory GABAergic brake on opioid antinociception. This study provides mechanistic insight into how Hsp90 inhibitors can be used to boost opioid antinociception and may have identified a novel inhibitory circuit of opioid antinociception.

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Poster

P406. Opioid Treatment

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Topic: D.03. Somatosensation – Pain

Title: Functional Consequences of Mu Opioid Receptor Pharmacogenomics

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Abstract: G-protein-coupled receptors (GPCRs) are a superfamily of 7-transmembrane protein receptors with diverse physiological functions in the human body. Targeted by >30% of all FDA approved drugs, GPCRs are critical targets of clinical intervention; however, human genome sequencing initiatives have revealed a number of high-frequency coding variations in drug-targeted GPCRs with uncharacterized pharmacological consequences. Recent evidence has highlighted that these mutations can fundamentally alter the intended pharmacology of the signal-ligand interaction and impact the GPCR transducerome profile, yet understanding the breadth of these variations is an emerging and still unmet need in clinical settings due to limitations in technology capable of translating pharmacogenomic impacts in-vitro to information of clinical significance. Among GPCRs, genetic variation in opioid receptors is of incredible pharmacogenomic significance due to their role in mediating neurotransmitter activity by both endogenous and exogenous ligand interactions. The potential hazards of uncharacterized mutations in opioid receptors is of particular importance with respect to the ongoing opioid

epidemic, as exogenous opioid compounds classically have analgesic properties yet pose a high risk for tolerance, dependence, and addiction in certain individuals. We are addressing this inscience with an optimized bioluminescence resonance energy transfer-based (BRET2) technology (TRUPATH) that is capable of methodically profiling GPCR pharmacological agents in real time. Our goal is to use TRUPATH to characterize the pharmacogenomic significance of human genetic variation in opioid receptors in-vitro and translate it into information that may be clinically relevant in understanding differences among opioid drug responses in patients. In this study, we assessed how common and previously unstudied mutations in the μ-opioid receptor alter the activity of endogenous and small molecule ligands. Drug- and dose-response activity was measured exclusively to the intended G-protein subfamily using robust tissue culture assays (N=3, n=4), and effects of opioid receptor mutations on the GPCR transducerome profile were analyzed and reported using standard controls and practices in the field of GPCR pharmacology. Preliminary interrogation of G-proteins that canonically bind to the μ-opioid receptors has shown that many high-frequency variants have altered receptor efficacy, potency, and transmutation of G-protein signaling identity, indicating that action of both endogenous and exogenous opioid compounds are perturbed by common μ-opioid receptor variants.

Disclosures: L. Lee: None. **A. Hauser:** None. **J. English:** None.

Poster

P427. Taste I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P427.01

Topic: D.05. Olfaction and Taste

Support: NIH/NIDCD grant R01 DC018733 (SDR, NC)

Title: Do sugars and artificial sweeteners activate identical subsets of gustatory sensory ganglion neurons?

Authors: *S. HAYOZ[1], G. DVORYANCHIKOV[1], W. M. CONNELLY[3], N. CHAUDHARI[1], *S. D. ROPER[2];
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Abstract: Sugars and artificial sweeteners activate the dimeric taste receptor, Tas1R2+Tas1R3, to elicit sweet taste. However, certain sugars such as glucose also stimulate taste cells by blocking K_{ATP} channels, following sugar uptake via SGLT1 and GLUT transporters and generation of ATP. Interestingly, independent of Tas1Rs, glucose uptake by taste cells initiates an important physiological reflex−cephalic phase insulin release (**CPIR**). Yet, the two transduction pathways (initiated by Tas1Rs or glucose transporters) co-exist in the same taste

receptor cells. This raises the question: how do certain sugars (e.g., glucose) activate an ascending pathway for perception (sweet) as well as initiate a medullary reflex (CPIR), whereas others (e.g., fructose) only evoke sweet perception? That is, do the two neural pathways diverge at the first central relay (neurons in the Nucleus of the Solitary Tract), or are there two distinct afferent sensory fiber types that innervate taste bud cells and transmit signals from the two transduction pathways? We used *in vivo* Ca^{2+} imaging of geniculate ganglion neurons in transgenic mice (n=68 neurons, 4 mice) that express GCaMP6s in these sensory neurons to test if separate geniculate ganglion neurons might subserve these two pathways. We tested (a) **fructose** and **sucralose** as activators only of Tas1Rs; (b) **glucose**, which is transduced by both pathways; and (c) **SolCarb**, a non-sweet oligosaccharide that activates only the K_{ATP} pathway. Concentration-response relations were obtained for each compound to determine isoeffective concentrations: 800 mM glucose, 800 mM fructose, 16 % SolCarb, and 1-2 mM sucralose. To obtain a broader view of the spectrum of taste-evoked responses in these geniculate ganglion neurons, we also tested a panel of 5 prototypic basic tastes: 10 mM citric acid (sour); 300 mM sucrose (sweet); 100 mM MSG + 1 mM IMP (umami); 250 mM NaCl (salt); and 1 µM cycloheximide + 0.3 mM quinine (bitter). Unexpectedly, only about half the geniculate neurons (32 out of 68 neurons) that responded to fructose, glucose, SolCarb, or sucralose also responded to sucrose. Moreover, glucose and SolCarb responses were consistently smaller (ΔF/F) and more transient than responses to sucrose and sucralose. Preliminary analyses suggest that a restricted subset of neurons may respond to glucose and SolCarb. Correlations across other sweeteners are in progress.

Disclosures: S. Hayoz: None. **G. Dvoryanchikov:** None. **W.M. Connelly:** None. **N. Chaudhari:** None. **S.D. Roper:** None.

Poster

P427. Taste I

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Program #/Poster #: P427.02

Topic: D.05. Olfaction and Taste

Support: Simons Society of Fellows
E.E. Just Fellowship

Title: Intergenerational inheritance of conditioned taste aversion

Authors: *C. W. LIFF[1], E. R. SHERMAN[1,3], E. C. JAEGER[1], R. AXEL[1,2], B. J. MARLIN[1,2]; [1]Zuckerman Inst., [2]Dept. of Neurosci., Columbia Univ., New York, NY; [3]Barnard Col., New York, NY

Abstract: Rationale: Following the Dutch Hunger Winter famine of 1944-1945, the children and grandchildren of survivors — who had not experienced this stress directly — exhibited elevated rates of both metabolic illness (e.g. obesity, diabetes) as well as adverse psychological outcomes (e.g. schizophrenia, anxiety). This phenomenon of physical or emotional trauma being transmitted to offspring via epigenetic modifications, called transgenerational epigenetic inheritance (TEI), is an intriguing mechanism by which a learned behavior in a parent can become an innate behavior in their offspring.
Dias and Ressler (2014) has demonstrated that pairing an odor with a foot shock leads to an increase in the number of olfactory sensory neurons expressing the receptor for the conditioned odor, in both the conditioned mice and their untrained offspring. This study examines whether a conditioned taste aversion (CTA) can also be inherited.
Methods: We utilize behavioral assays to manipulate flavor preferences in male C57BL/6J mice (F0) and their offspring (F1). To produce CTA, we pair consumption of 5.5mM saccharin with injection of either lithium chloride (LiCl), which induces nausea, or PBS. The treated males are mated with female C57BL/6J mice and removed from the cage before the litter is born to prevent father-offspring interaction. The males' saccharin preferences are tested in a 10-minute 2-bottle test, measuring how much time they spend drinking from saccharin v. water. The offspring are exposed to 5.5mM saccharin for 5h to eliminate neophobia effects, and later given the same 2-bottle test.
Results: Our results demonstrate a highly significant difference ($p<0.0001$) between the saccharin preferences of F0 PBS-injected and LiCl-injected male mice, with PBS mice exhibiting a preference for saccharin and LiCl mice showing a strong aversion (F0 PBS n = 12, F0 LiCl n = 11). We have also found a very significant difference ($p=0.0002$) between the saccharin preferences among the offspring of PBS-paired fathers and LiCl-paired fathers, with the F1 of LiCl males demonstrating an innate aversion to what is innately appetitive in controls (F1 PBS n = 12, F1 LiCl n = 23).
Conclusions: The brain's memory of trauma is much more complex than the memory of a single sense during a traumatic event. While instances of TEI have been demonstrated in the olfactory system in mice, there is limited evidence of this phenomenon in other sensory systems. This project elucidates whether this mechanism of epigenetic inheritance is also inherent in the gustatory system. Furthermore, an understanding of how to intervene when these adaptations no longer benefit the offspring would be of immense translational value.

Disclosures: C.W. Liff: None. **E.R. Sherman:** None. **E.C. Jaeger:** None. **R. Axel:** None. **B.J. Marlin:** None.

Poster

P427. Taste I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P427.03

Topic: D.05. Olfaction and Taste

Title: Integration and segregated processing in the Drosophila taste circuit

Authors: J. U. DEERE, H. A. UTTLEY, N. MARTINEZ SANTANA, ***A. V. DEVINENI**; Zuckerman Mind Brain Behavior Inst., Columbia Univ., New York, NY

Abstract: The taste system is essential for regulating feeding. Sweet-sensing neurons promote the consumption of sugar, while bitter-sensing neurons inhibit feeding to prevent the ingestion of potential toxins. In Drosophila, each taste modality comprises multiple channels of sensory input - neurons that reside in different organs or express different combinations of taste receptors. How are these multiple channels of input translated into behavioral output? One possibility is that different input channels converge in the brain and elicit the same behaviors, reflecting an "integrative model". Alternatively, different input channels may activate separate pathways to regulate distinct behaviors, a "segregated processing" model.
We first used optogenetic activation to characterize how sweet and bitter inputs influence behaviors beyond feeding. Activating sweet- or bitter-sensing neurons elicited opposing effects on locomotion, positional preference, and associative learning. Next, we activated different subsets of bitter sensory neurons. Each neuronal subset influenced multiple behaviors and each behavior was affected by multiple subsets, with the strongest effects observed only when all bitter neurons were activated. These results support an integrative model in which multiple channels of bitter input are integrated to regulate a diverse set of behaviors.
To test whether this integration occurs early in the taste circuit, we used trans-synaptic tracing to label downstream second-order bitter neurons that receive input from bitter sensory cells. These experiments revealed multiple types of second-order bitter neurons projecting to distinct brain regions. We imaged the activity of one type and found that it integrates bitter input from multiple organs. Optogenetic manipulations showed that these neurons regulate feeding, but not other behaviors such as locomotion, preference, and learning. Thus second-order neurons integrate across multiple channels of bitter input, but have segregated outputs that regulate distinct behaviors. The integration of inputs enables the circuit to pool sensory information, whereas segregated outputs could allow for different behaviors to be differentially modulated by context or state.

Disclosures: J.U. Deere: None. **H.A. Uttley:** None. **N. Martinez Santana:** None. **A.V. Devineni:** None.

Poster

P427. Taste I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P427.04

Topic: D.05. Olfaction and Taste

Support: NIMH Grant ZIA MH002588-26

Title: Human similarity judgements of appetizing foods include estimates of sweetness and caloric density

Authors: *M. CARRINGTON[1], A. LIU[1], J. AVERY[1], C. ZHENG[2], L. MUTTENTHALER[3], F. PEREIRA[2], A. MARTIN[1];
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Abstract: Food-related scientific studies often select food items to include based on specific criteria, such as relative levels of fat and sugar content, and use these categorizations for further analyses. While they frequently make intuitive sense, it is unclear whether these lab-generated groupings align with the ways in which individuals naturally compare different foods. Hebart et al., (2020) used a simple 'odd-one-out' triplet task to collect similarity judgements of a wide variety of common objects. From these data, they were able to identify the most salient dimensions underlying the similarity judgements of these objects and used them to create categories. In our study, participants on Amazon Mechanical Turk performed this "odd-one-out" triplet task on a variety of food images. We used 36 images in total that had been selected to be evenly distributed among 4 categories based on high or low levels of fat and sugar (high fat high sugar, high fat low sugar, etc). Out of 7,140 possible triplets, each participant rated 20. We collected two full sets of triplet data from a total of 487 unique participants. The similarity matrices of these two sets were highly similar ($r = 0.91$, $p < 0.001$), thus allowing us to combine the two sets. Multi-dimensional scaling of the resulting similarity matrix and k-means clustering revealed five emergent food groupings: sweets, fats, starches, fruits, and vegetables. A subsequent Principal Components Analysis indicated that the first two components accounted for approximately 75% of the variability of the data. The first component was significantly related to the caloric density of the foods, rather than fat content specifically ($r = 0.88$, $p < 0.001$), while the second component was significantly related to sugar content ($r = -0.63$, $p < 0.001$). A Non-Negative Matrix Factorization (NNMF) analysis of f sparse non-negative embeddings learned from the triplet data revealed a similar categorization of the foods used in our dataset. Our results suggest that naturalistic categorizations may differ from lab-generated groupings and that these differences should be considered and explored when developing methods for scientific food studies. Avery et al (2021) previously established that it is possible to use fMRI to decode the inferred taste of food pictures within cortical taste-responsive regions. Future studies might explore whether a similar decoding approach could be used to identify the neural substrates associated with the emergent food categories that we identified.

Disclosures: M. Carrington: None. **A. Liu:** None. **J. Avery:** None. **C. Zheng:** None. **L. Muttenthaler:** None. **F. Pereira:** None. **A. Martin:** None.

Poster

P427. Taste I

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Program #/Poster #: P427.05

Topic: D.05. Olfaction and Taste

Support: R01DC018227

Title: The role of cortical D1R-expressing neurons in taste-based sensorimotor transformations

Authors: *J. CHEN, A. FONTANINI;
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Abstract: To survive, animals must use gustatory stimuli to guide oromotor behaviors like licking and chewing. The anterior-lateral motor cortex (ALM) is involved in the genesis of sensorimotor decisions related to licking. Although licking and taste are invariably linked, it remains unknown if ALM plays a role in taste-based decisions, and how the underlying circuits are modulated during this process. One candidate mechanism for taste-based sensorimotor transformations involves dopamine transmission through D1 receptor (D1R). Blockade of D1R signaling within ALM affects both sensory cue-evoked responses and motor planning of licking, suggesting a potential role for D1R-expressing (D1R+) neurons in sensorimotor decisions related to licking. We tested the hypothesis that D1R+ neurons in ALM exhibit patterns of activity that represent taste cues and preparation of licking, and that these specific and distinct representations are required to drive lick decisions during a taste-guided 2-alternative choice (2-AC) task. In this task, mice must use distinct taste cues to guide different licking actions (lick left vs lick right) after a delay period. To monitor activity of D1R+ neurons during the task, 2-photon calcium imaging was performed using transgenic D1R reporter mice to identify and record populations of D1R+ neurons located in the superficial layers (L1, L2/3, z < 350 um) and deep layers of ALM (L5 z > 350 um) of ALM. We found that individual D1R+ neurons in both laminar locations exhibit responses to specific taste cues, preparatory activity predicting licking direction and coding of reward presentation during the response period. The majority of taste and lick direction coding occurred during the sampling and delay periods of the task, respectively. D1R+ neurons in the deep layers showed weaker tuning for taste and lick direction during these behavioral periods, suggesting differential coding of task variables based on cell-type and laminar location. To determine if and when activity of D1R+ neurons, in both superficial and deep layers, is required to generate taste-based lick decisions, we will rely on optogenetics to bilaterally silence D1R+ neurons during different behavioral periods of the 2-AC task. We expect silencing D1R+ neurons during the delay will produce motor deficits in licking execution and duration as found previously. In addition, we anticipate a decrease in task performance when D1R+ neurons are inhibited during the sampling or delay periods of the task, indicative of their critical role in taste-guided licking. Altogether, these findings will provide novel understanding of the link between gustatory decision-making and neurons expressing D1Rs.

Disclosures: J. Chen: None. **A. Fontanini:** None.

Poster

P427. Taste I

Location: Virtual Only

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Program #/Poster #: P427.06

Topic: D.05. Olfaction and Taste

Support: Jane Coffin Childs Fellowship (Z.Y.)
NIH grant R01GM128209 (K.S.)

Title: Serotonergic neurons translate taste detection into internal nutrient regulation

Authors: *Z. YAO, K. SCOTT;
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Abstract: The nervous and endocrine systems coordinately monitor and regulate nutrient availability to maintain energy homeostasis. Sensory detection of food regulates internal nutrient availability in a manner that anticipates food intake, but sensory pathways that promote anticipatory physiological changes remain unclear. Here, we identify serotonergic (5-HT) neurons as critical mediators that transform gustatory detection by sensory neurons into the activation of insulin-producing cells and enteric neurons in *Drosophila*. One class of 5-HT neurons responds to gustatory detection of sugars, excites insulin-producing cells and limits consumption, suggesting that they anticipate increased nutrient levels and prevent overconsumption. A second class of 5-HT neurons responds to gustatory detection of bitter compounds and activates enteric neurons to promote gastric motility, likely to stimulate digestion and increase circulating nutrients when food quality is poor. These studies demonstrate that 5-HT neurons relay acute gustatory detection to divergent pathways for longer-term stabilization of circulating nutrients.

Disclosures: Z. Yao: None. **K. Scott:** None.

Poster

P427. Taste I

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Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P427.07

Topic: D.05. Olfaction and Taste

Support: NIH-SC2-GM130411

Title: Taste Response Profiles of Nodose/Petrosal Ganglia Neurons

Authors: *B. FOWLER, S. HUMAYUN, L. MACPHERSON;
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Abstract: In vivo calcium imaging of the geniculate ganglion has provided a way to categorize the response profiles of individual neurons to taste stimuli. Neurons of the geniculate ganglion innervate the fungiform papillae (FP) of the anterior tongue via the chorda tympani. The circumvallate (CV) papillae in the posterior tongue are innervated through the glossopharyngeal nerve by a separate population of neurons housed in the nodose/petrosal ganglion complex. Here we used in vivo calcium imaging approaches to determine the responses of nodose/petrosal neurons to taste stimuli, and to determine if this population differs from the geniculate ganglion. To image the activity of geniculate and nodose/petrosal neurons to taste stimuli, we used transgenic animals of both sexes expressing GCaMP6s under the Snap25 promoter. We surgically exposed the ganglia and imaged the GCaMP fluorescence changes while perfusing the tongue with a panel of taste stimuli. Comparing the nodose/petrosal taste response profiles to those obtained from the geniculate ganglion showed significant differences in the proportion of neurons responding to some classes of taste stimuli. In the nodose/petrosal, a smaller proportion of neurons respond to sweet, while larger percentages of neurons respond exclusively to bitter or umami tastants. No significant differences in the proportions of neurons responding to sour, or multiple stimuli were observed. Additional calcium imaging experiments were conducted to determine if high salt concentrations recruit responses from bitter and sour responding nodose/petrosal neurons. We find that a greater proportion of taste-activated nodose neurons respond to bitter and umami tastants compared to those of the geniculate ganglion. These data support previous experiments showing enhanced responses to bitter and umami stimuli in glossopharyngeal nerve recordings. Further research will address whether these differences in taste responses from the front and back of tongue are important for signaling reward or aversion, if they serve as preparatory signals to digestion, or a combination.

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Poster

P427. Taste I

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Program #/Poster #: P427.08

Topic: D.05. Olfaction and Taste

Support: Fidelity Charitable Nancy Adams and Scott Schoen Fund
Kraig and Linda Kupiec Family Trust

Title: Ancillary autonomic changes accompanying mechanical neuromodulation for migraine and trigeminal neuropathy pain

Authors: *V. LUBERA[1], R. K. HARPER[1], D. N. SNODGRASS[2], R. L. MERRILL[3], E. SAUERLAND[4], R. M. HARPER[1];
[1]Neurobio., [2]Auditory Clin., [3]Orofacial Pain, UCLA, Los Angeles, CA; [4]Physiol. and Cell Biol., Univ. of Nevada, Reno, NV

Abstract: Non-invasive, mechanical neuromodulation of sensory fields of cranial nerves V, VII, IX, and X, and cervical nerves C2 and C3 sited near the auditory canal reduces acute migraine and trigeminal neuropathy pain. Several of these cranial nerves are accompanied by autonomic fibers which are also activated by neuromodulatory stimulation. While evaluating vibratory stimulation effectiveness, autonomic responses appeared in addition to pain resolution; these included heart rate slowing, increased respiratory-related heart rate variation, and blood pressure normalization. However, other autonomic aspects appeared, principally related to secretory gland outflow; we evaluated that expression.
All procedures were approved by the UCLA Institutional Review Board. Neuromodulatory devices consisted of bilateral silicon impressions of the auditory canals, each containing a vibration device which reached the cranial and cervical sensory fields within the auditory canals and pinnae. Subjects with diagnosed migraine or trigeminal neuropathy pain (N=56, female=37, male=18) were referred by UCLA or Mayo Clinic neurologists or pain specialists and subjected to 5 min sham, 15 min low, and 10 min high amplitude vibration, and a second 10 min sham vibration to affected nerves. Pain was reduced (Mean= 5.7 pre-stimulation, 1.8 post-stimulation ($p < 0.0001$) on (0-10 pain scales).
Increased activation of secretory tissue appeared, particularly nasal, oral, and lacrimal secretions. Of the 56 subjects evaluated, over half of subjects showed one or more increased secretions; the most common being increased oral saliva expression, followed by enhanced nasal discharge and lacrimal outflow. The salivary secretions, unlike lacrimal actions, were sustained for several days.The oral secretions were perceived as beneficial; trigeminal neuropathy pain is commonly accompanied by xerostomia which is associated with loss or altered taste, disturbed olfaction, increased dental caries, demineralization of teeth, opportunistic infections, impaired swallowing, and bad breath. The intervention for migraine was accompanied by enhanced parasympathetic-regulated secretory expression, with oral secretions more frequently affected. The preferential effects on saliva most likely derive from the major influences on trigeminal sensory fields on the pinnae and auditory canals; the trigeminal fibers are accompanied by autonomic nerves targeting ganglia of the head. The intervention offers a low-cost, drug-free, and simple means to overcome xerostomia.

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Poster

P436. Auditory Processing: Circuit Function and Dysfunction

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P436.01

Topic: D.06. Auditory & Vestibular Systems

Support: Predoctoral Research Fellowship for Central Auditory Processing Disorders, Autism Speaks, Royal Arch Masons #11100
NIH National Institute of Mental Health R01- 5R01MH106656-05

Title: Methyl CpG Binding Protein 2 Knockout in Auditory Cortex Parvalbumin Cortical Interneurons Alters Temporal Structure of Responses to Ultrasonic Vocalizations

Authors: *D. D. RUPERT[1,2], A. H. PAGLIARO[1], S. D. SHEA[1];
[1]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [2]Med. Scientist Training Program, Stony Brook Univ., Stony Brook, NY

Abstract: Rett Syndrome (RTT) is a pervasive, neurodevelopmental disorder previously classified as a syndromic form of Autism Spectrum Disorder. Unlike ASDs, RTT is monogenic- caused by loss of function mutations in the X-linked gene methyl CpG binding protein 2 (*MeCP2*). *MeCP2* codes for the protein by the same name, a ubiquitously expressed transcription factor with a known role in the plasticity of mature synapses. In humans with Rett syndrome, and in corresponding animal models, tissues comprise a mosaic pattern of cells expressing normal MeCP2 and those with absent expression. Whether certain cell-types within that mosaic contribute more or less to phenotypic characteristics is unknown. We previously reported that adult animals that are heterozygous for *Mecp2* mutation demonstrate impaired auditory processing characterized by altered responses of auditory cortex (ACtx) cells to ultrasonic vocalization (USV) playbacks, and upregulation of anti-plasticity molecular markers by those cells. Here we hypothesized that aberrant audition may result in the relative dependence of ACtx, inhibition interneurons on MeCP2. **That is, our goal was to determine the contribution of cell-type specific *Mecp2* mutation to disruptions of auditory cortical circuitry and plasticity that degrade social vocal perception.** To achieve this goal, we used conditional (cell-type restricted) *Mecp2* mutant mouse models in combination with a pup retrieval assay- an ethologically relevant, auditory-dependent behavior; pup retrieval is cued by ultrasonic vocalizations (USVs)- high-pitched distress calls emitted by isolated infants. We report the consequences of cell-type restricted *Mecp2* knockdown on that behavior, single-unit *in vivo* neural circuit activity of excitatory and inhibitory populations, and calcium imaging of ACtx, parvalbumin cells. Our findings expand our understanding of ACtx plasticity in neurotypically developing animals, cell-type specific contributions to that plasticity, and how it is affected by *Mecp2* mutation.

Disclosures: D.D. Rupert: None. **A.H. Pagliaro:** None. **S.D. Shea:** None.

Poster

P436. Auditory Processing: Circuit Function and Dysfunction

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P436.02

Topic: D.06. Auditory & Vestibular Systems

Title: Age-related changes in excitatory and inhibitory intra-cortical circuits in mouse primary auritory cortex

Authors: *B. XUE[1], X. MENG[2], J. KAO[3], P. KANOLD[1];
[1]Johns Hopkins Univ., Baltimore, MD; [2]Biol., Univ. of Maryland, College Park, MD; [3]Physiol., Univ. of Maryland, Baltimore, MD

Abstract: A common impairment in aging is age-related hearing loss (presbycusis), which manifests as impaired spectrotemporal processing. Aging is accompanied by alteration in normal inhibitory (GABA) neurotransmission, and changes in excitatory (NMDA and AMPA) synapses in the auditory cortex (ACtx). However, the circuit mechanisms responsible for age-related hearing loss remain unknown. Here we investigated how auditory cortical microcircuits change with age. We performed laser scanning photostimulation (LSPS) combined with whole-cell patch clamp recordings from Layer (L) 2/3 cells in primary auditory cortex (A1) in young adult {postnatal day (P) 30-45} and aged (P370-P443) C57BL/6J mice. We found that L2/3 cells in aged animals display functional hypoconnectivity of both excitatory and inhibitory circuits. Compared to cells from young adult mice, cells from aged mice have fewer excitatory connections with weaker connection strength. Whereas young adult and aged animals have similar amounts of inhibitory connections, the strength of local inhibition is weaker in the aged group. We confirmed these results by recording
miniature excitatory (mEPSCs) and inhibitory synaptic currents (mIPSCs), which showed a reduction in mEPSC amplitude and frequency but only a reduction in mIPSC amplitude in the aged group. These results suggest a specific reduction in excitatory and inhibitory intralaminar cortical circuits in aged mice compared with young adult animals. We speculate that these unbalanced changes in cortical circuits contribute to the functional manifestations of age-related hearing loss.

Disclosures: B. Xue: None. **X. Meng:** None. **J. Kao:** None. **P. Kanold:** None.

Poster

P436. Auditory Processing: Circuit Function and Dysfunction

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Program #/Poster #: P436.03

Topic: D.06. Auditory & Vestibular Systems

Support: NIH Grant DC13074

NIH Grant DC17267

Title: The role of fragile X mental retardation protein in cellular and synaptic dynamics of developing auditory circuits

Authors: *X. YU, Y. WANG;
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Abstract: The establishment of central sensory systems is influenced by afferent experience during development. In the auditory system, hearing loss at young ages leads to neuronal cell loss and circuit reorganizations, yet the responsible molecular players have not been identified. We addressed this question by investigating the neuropathology of fragile X syndrome (FXS), a leading genetic cause of neurodevelopmental disorders with prominent auditory dysfunction. FXS results from a loss of fragile X mental retardation protein (FMRP) encoded by Fmr1 gene. Studies of Fmr1 knockout (KO) rodents revealed abnormal circuit development and altered neuronal plasticity in the auditory system. However, little is known about how FMRP regulates neuronal integrity and synaptic connectivity in response to changes in afferent input. This study aimed to determine the consequences of FMRP absence in auditory processing, with and without intact afferent input, during early development. We first examined afferent-regulated neuronal integrity in the ventral cochlea nucleus (VCN), the first brain station of auditory afferent input. In wildtype (WT) mice, VCN exhibited a critical period (CP) of neuronal cell loss following afferent deprivation, showing 30% cell loss if the deprivation occurred within the first postnatal (P) 11 days but no cell loss if it was induced later. We found the presence of a CP and a comparable level of cell loss within the CP in *Fmr1* KO mice. However, the timing of CP closure was significantly delayed in *Fmr1* KO mice, evident by substantial cell loss following afferent deprivation at P14, an age beyond the CP in WT. Next, we examined synaptic development and plasticity in the medial nucleus of the trapezoid body (MNTB), which receives the excitatory input from the VCN and serves as an inhibitory station in the auditory brainstem. In WT mice, we discovered a distinct topographic gradient in the level of several key synaptic proteins before P7 and this gradient was not significant after hearing onset at P10-12. *Fmr1* KO mice exhibited a delayed progress of this developmental event. Following afferent deprivation, WT mice showed a significant increase in the density of inhibitory synapses on MNTB neurons. This response was diminished in *Fmr1* KO mice, demonstrating a requirement of FMRP for inhibitory synaptic plasticity. Finally, using auditory brainstem responses as a functional assessment of hearing ability, we revealed an impair hearing sensitivity in *Fmr1* KO mice at P14. Together, these findings demonstrate that FMRP is required for selective aspects of afferent-regulated neuronal dynamics in the auditory brainstem at cellular, connective, and functional levels.

Disclosures: X. Yu: None. **Y. Wang:** None.

Poster

P436. Auditory Processing: Circuit Function and Dysfunction

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Program #/Poster #: P436.04

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NIH U54 MD007601

Title: Faulty GABAergic inhibition and perineuronal net integrity in the inferior colliculus of subjects with schizophrenia

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Abstract: Aberrant processing of auditory stimuli is a prominent feature of schizophrenia (SZ). Prior studies have chronicled histological abnormalities in the auditory cortex of SZ subjects, but whether deficits exist at upstream, subcortical levels has yet to be established. En route to the auditory cortex, ascending information is integrated in the inferior colliculus (IC), a highly gamma-aminobutyric acid (GABA) ergic midbrain structure that is critically involved in auditory processing. The IC contains a dense population of parvalbumin-immunoreactive interneurons (PVIs), a cell type characterized by increased metabolic demands and enhanced vulnerability to oxidative stress. During development, PVIs are preferentially surrounded by perineuronal nets (PNNs), specialized extracellular matrix structures that promote redox homeostasis and excitatory/inhibitory balance. Moreover, in SZ, deficits in PVIs, PNNs, and the GABA synthesizing enzyme, glutamic acid decarboxylase (Gad67), have been extensively documented in cortical regions. Yet, whether similar impairments exist in the IC is currently unknown. Thus, we compared IC samples of age- and sex-matched pairs of SZ and unaffected control subjects. SZ subjects exhibited lower levels of Gad67 immunoreactivity and a decreased density of PVIs and PNNs within the IC. These findings provide the first histological evidence of IC GABAergic abnormalities in SZ and suggest that SZ-related auditory dysfunction may stem, in part, from altered IC inhibitory tone.

Disclosures: V.W. Kilonzo: None. **R.A. Sweet:** None. **J.R. Glausier:** None. **M.W. Pitts:** None.

Poster

P436. Auditory Processing: Circuit Function and Dysfunction

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Program #/Poster #: P436.05

Topic: D.06. Auditory & Vestibular Systems

Support: DOD W81XWH1910017

Title: Vip+ interneurons modulate tinnitus-related hyperactivity of A1 L5 pyramid neurons

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Abstract: Tinnitus, commonly referred to as ringing in the ear, is believed to reflect maladaptive plastic changes at various levels of the central auditory pathway. Primary auditory cortex (A1) houses complex microcircuitry of excitatory and inhibitory neurons, responsible for higher order processing and integration of auditory information. Deeper layers of A1 are believed to be engaged in top-down modulation of ascending signals, as well as the cortico-cortical auditory processing. The net excitability of Layer (L) 5 pyramid neurons (PNs) is modulated by a delicate balance of excitation, inhibition and disinhibition. Vasoactive intestinal polypeptide positive (VIP+) interneurons serve as a key regulator of PN excitability via disinhibitory mechanisms. The impact of tinnitus on the intrinsic properties of A1 L5 PNs and VIP+ neurons is the focus of the present study. Using a well-established condition-suppression animal model of tinnitus, we hypothesize that: 1) changes in intrinsic properties and neurotransmission underpin the tinnitus-related hyperactivity of A1 L5 PNs; and 2) tinnitus-related altered VIP+ neuronal function indirectly contributes to PN hyperactivity underpinning tinnitus pathology. In the present study, wild type and VIP*TdTomato Long Evens (LE) rats were unilaterally sound exposed for 1 hour, to an octave band noise centered at 16 kHz frequency. Three months following noise exposure, most exposed rats show psychophysical evidence of tinnitus which persists throughout their lifetime. Both group of animals were classified as control and exposed tinnitus based on their exposure history and tinnitus score. Animals were used to perform whole cell patch clamp recordings from A1 L5 PNs and VIP+ neurons, and spontaneous and evoked responses were recorded from these neurons. A1 L5 PNs showed a significant tinnitus-related increase in spontaneous and evoked, pre- and post-synaptic excitatory currents. Additionally, we observed a tinnitus-related decrease in spontaneous and evoked, pre- and post-synaptic inhibitory currents. Increased glutamatergic excitability was mostly polysynaptic, suggesting involvement of local microcircuitory in tinnitus pathology. VIP+ neurons in A1 were found to be significantly depolarized, with smaller rheobase current and increased excitability in tinnitus animals. VIP+ neurons when activated, are known to depolarize the PNs, and increase their excitability. In conclusion, we report that changes in intrinsic properties and neurotransmission underpin tinnitus related hyperactivity of A1 L5 PNs, and these changes, in part, reflect tinnitus-related maladaptive plastic changes in VIP+ neuronal function.

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Poster

P436. Auditory Processing: Circuit Function and Dysfunction

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Program #/Poster #: P436.06

Topic: D.01. Sensory Disorders

Support: DOD: USA | MEDCOM | CDMRP: W81XWH-18-1-0135

Title: Comparison of Active Avoidance and GPIAS for Tinnitus Assessment in Mice

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Abstract: Tinnitus, a phantom perception of sound with no external auditory stimulus, is experienced by over 15 million Americans. Research towards new treatments for tinnitus can be expensive and time-consuming due to the behavioral training needed to assess laboratory animals for tinnitus. There are several different behavioral tests for tinnitus, but little direct comparison of these tests. CBA/CaJ mice were trained in two types of tinnitus behavioral tests: the gap-induced pre-pulse inhibition of acoustic startle (GPIAS) and an active avoidance (AA) test (n=19). Additional mice were only trained in one test (AA n=8, GPIAS n=18). Tinnitus was induced in awake mice by a unilateral continuous sound exposure using a 2 kHz wide, 16 kHz centered noise at 113- or 116-dB SPL. Behavioral testing was repeated 8 weeks after sound exposure to determine tinnitus frequency. Of sound exposed mice trained in both tests, the majority (63%, n=12) showed tinnitus behavior in at least one of the two tests. Evidence of tinnitus was found in 50% (n=4) of mice trained only in AA and 33% (n=6) of GPIAS-tested mice. When mice were tested with both methods, 37% (n=7) did not show any evidence of tinnitus behavior. However, 52% (n=10) were positive in AA, while only 26% (n=5) were positive in GPIAS. Only 15% (n=3) mice were positive in both tests and only 5% (n=1) were positive in both tests at the same tinnitus frequency. Spontaneous firing rates of neurons in the inferior colliculus were collected from the tinnitus positive and negative mice. Neurons were sorted based on the characteristic frequency (CF), and the spontaneous activity of neurons with the CF at 16 kHz was compared to those with CFs above and below. In mice tested in AA, if the CF was higher than the exposure frequency, the spontaneous firing was higher in tinnitus-positive mice than in tinnitus-negative mice. This pattern was not evident in mice tested with GPIAS. These results suggest that the GPIAS and AA tests are not equally valid for diagnosing tinnitus in mice, as they do not often show the same frequencies of deficit. Furthermore, increased spontaneous rate, shown to be increased in the IC in tinnitus mice (Berger & Coomber, 2015) is associated with the AA behavioral test. This suggests that the AA test may be a more reliable behavioral measure of tinnitus.

Disclosures: E. Fabrizio-Stover: None. **A.L. Burghard:** None. **C.M. Lee:** None. **D.L. Oliver:** None.

Poster

P436. Auditory Processing: Circuit Function and Dysfunction

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Program #/Poster #: P436.07

Topic: D.01. Sensory Disorders

Title: A first-in-human study of OTO-413, an intratympanic sustained-exposure formulation of BDNF, for the treatment of hearing loss

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Abstract: Synaptic connections between inner hair cells (IHCs) and type 1 spiral ganglion neurons (SGNs) in the cochlea may be lost after noise exposure and with aging, even without notable hair cell damage. Loss of cochlear synapses in the absence of changes in auditory thresholds has been proposed as a mechanism of speech-in-noise hearing impairment and may be one of the earliest manifestations of sensorineural hearing loss. Treatment with brain-derived neurotrophic factor (BDNF) repairs synaptic processes and restores hearing function in animal models. OTO-413, a thermo-sensitive formulation of BDNF which provides sustained exposure of BDNF to the cochlea after intratympanic (IT) administration, is under clinical evaluation for the treatment of hearing loss. This was a randomized, double-blind, placebo-controlled Phase 1/2 study. Men and women aged 21-64 years were enrolled between December 2019 and August 2020. Pure tone thresholds ranged from normal levels to moderately severe hearing loss (PTA of ≤ 70 dB at 1, 2, and 4 kHz). Subject's self-reported difficulty hearing in noise was confirmed by Digits-in-Noise (DIN) test scores greater than -12.5 dB signal-to-noise ratio (SNR) in the study ear. Four ascending dose-cohorts of at least 8 subjects each received a single IT injection of OTO-413 or placebo. Safety and hearing function were monitored over a 12-week follow-up. Hearing assessments included DIN, Words-in-Noise (WIN) test, and American English Matrix test (AEMT). OTO-413 was well-tolerated across all dose cohorts (n = 29) with similar frequency of adverse events compared to placebo (n = 10). There were no serious adverse events. Six of 9 (67%) subjects treated with the highest dose of OTO-413 (0.30 mg) showed a clinically meaningful improvement on at least one of the three speech-in-noise tests at both Day 57 and 85 versus 0 of 8 (0%) for placebo. A clinically meaningful improvement was defined as a minimum change of -3 dB SNR (DIN) or -2 dB SNR (WIN and AEMT). Performance on the sentence-based AEMT favored OTO-413, with 4 of 9 (44%) OTO-413 subjects experiencing a clinically meaningful improvement at both Day 57 and 85 compared to 0 of 7 (0%) placebo subjects at any single timepoint. These results demonstrate safety of OTO-413 and suggest therapeutic benefit

over placebo in a small sample size. These findings support further clinical development of OTO-413 for the treatment of hearing loss.

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Poster

P436. Auditory Processing: Circuit Function and Dysfunction

Location: Virtual Only

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Program #/Poster #: P436.08

Topic: D.01. Sensory Disorders

Title: Initial clinical evaluation of OTO-313 given as a single intratympanic injection in patients with moderate to severe, persistent tinnitus

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Abstract: Tinnitus, a perception of sounds without a correlated external auditory stimulus, is widely prevalent and often associated with distress. Excessive activation of NMDA receptors within the hair cell complex due to noise or other cochlear insults may be of particular importance in altering activity of the auditory nerve and generating subjective tinnitus. OTO-313 is a lipid-based formulation of the potent and selective NMDA receptor antagonist gacyclidine that provides sustained exposure to the cochlea after a single intratympanic (IT) injection. The safety, plasma pharmacokinetics (PK), and exploratory efficacy of OTO-313 was evaluated in a randomized, double-blind, placebo-controlled, Phase 1/2 study. An initial safety and plasma PK cohort of 8 patients with tinnitus received a single IT injection of either 0.11 mg OTO-313 or placebo (PL). A follow-on exploratory efficacy cohort included 35 patients with unilateral tinnitus of moderate to severe intensity (score of $\geq$ 25 on the Tinnitus Functional Index [TFI]) and a duration of tinnitus of 1 to 6 months since onset. Following a 2-week lead-in period, patients received a single IT injection of either 0.32 mg OTO-313 or PL (1:1). Safety and exploratory efficacy (TFI, daily ratings of tinnitus loudness and annoyance, and PGIC) were evaluated over an 8-week follow-up period. OTO-313 was well-tolerated with a lower incidence of adverse events than placebo. In the exploratory efficacy cohort, the mean TFI reduction from baseline trended in favor of OTO-313 at each timepoint (Day 15: -9.3 points OTO-313 vs -4.1

points PL; Day 29: -9.4 points OTO-313 vs -6.6 points PL; Day 57: -12.9 points OTO-313 vs -4.3 points PL). A clinically meaningful, 13-point improvement on the TFI was observed in 43% (6/14) of OTO-313 patients at both Day 29 and Day 57 versus 13% (2/16) of PL patients (*adhoc* p-value < 0.05). Treatment with OTO-313 led to reduction in the daily ratings of tinnitus loudness and annoyance as well as improved PGIC scores. Among responders, strong correlation was observed between the various endpoints in favor of OTO-313. Plasma concentrations of gacyclidine were below the limit of assay quantitation (≤ 0.1 ng/mL) confirming limited systemic exposure. These findings demonstrate safety and exploratory efficacy of OTO-313 and support further clinical development for the treatment of tinnitus.

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Poster

P436. Auditory Processing: Circuit Function and Dysfunction

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Topic: D.01. Sensory Disorders

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Title: Ultrahearing: stimulation with complex signals via ultrasound for a new type of hearing technology

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Abstract: Ultrasound stimulation (US) is an exciting new technique to noninvasively modulate neural activity with high spatial precision. However, recent studies have demonstrated that US, when coupled to the subject, induces activation of the auditory system. Guo et al. (Neuron, 2018) have provided convincing data that the method of activation is via a fluid pathway, in which the ultrasound waves induce vibrations of the cerebrospinal fluid and travel into the cochlea via the cochlear and vestibular aqueducts. This finding suggests that the stimulus will have different

characteristics from traditional air- and bone-conduction stimuli. Recent work in our lab has demonstrated that amplitude-modulated ultrasound signals can effectively encode and transmit information relevant for sound perception. However, these experiments have been limited to pure-tone modulations, and thus the range and information capacity of ultrasound is still unknown.
In this study, we investigated the neural activity in response to complex signals transmitted via bone-, air-, and fluid-conduction (ultrasound). For this purpose, we used a rich library of 11 different guinea pig vocalizations that have been used in previous published research. To create the ultrasound stimuli, vocalizations were upsampled, the envelope was extracted using the Hilbert transform, and then used to modulate a 220 kHz sinusoid carrier. Neural activity was recorded using a two-shank 32-channel NeuroNexus electrode array placed in the inferior colliculus (ICC) of five young and female, anesthetized guinea pigs. A B-81 bone conduction device was coupled to the animal as described by Curthoys et al.(Exp Brain Res, 2006). In response to the stimuli, we created post-stimulus time histograms (PSTHs) to assess multi-unit activity and identify the temporal behavior of the neural responses. We utilized the Earth Mover's Distance metric to compare different PSTHs.
Our results demonstrate that ultrasound can encode complex signals. ICC activity reliably followed the envelope, including fast temporal transitions, particularly ICC regions that encode for high frequencies. PSTHs in response to ultrasound and air stimuli have a high degree of similarity, when the same signal is presented via both modalities; however, PSTHs in response to bone conduction differed significantly. Further analyses will explore and quantify the differences between the response of these stimulation modalities. Better understanding of ultrasound stimuli on the auditory system can help guide the development of next-generation hearing devices in addition to combination technologies levering different stimulation modalities.

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Poster

P436. Auditory Processing: Circuit Function and Dysfunction

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Title: Fibre-specific white matter structural abnormalities differentiate acoustic neuroma patients with one-sided hearing loss from individuals with normal hearing

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Abstract: Objectives
Acoustic neuroma (AN) tumours are a chief cause of one-sided hearing loss in adults. One-sided hearing loss results from auditory nerve compression by the tumour at the pontine level of the brainstem. Treating one-sided hearing loss will require identifying targets of white matter nerve fibre abnormalities above the tumour site. We used a state-of-the-art technique called a "fixel-based analysis" to identify fibre-specific, or "fixel"-level differences in white matter structure between AN patients with one-sided hearing loss and individuals with normal hearing (NH). We hypothesized wide-spread abnormalities in white matter structure in the AN group.
Methods
Diffusion-weighted MRI data was collected from left- and right-sided AN patients treated at the Toronto Western Hospital, and NH healthy controls tested at Toronto Western. The fixel-based analysis was used to identify microstructural differences in nerve fibre density (FD), macrostructural differences in fibre cross-section (FC), and changes in a combined metric of density and cross-section (FDC) between groups. Pure-tone audiometry (PTA) scores were used to identify ipsilateral and contralateral hearing thresholds for each AN patient.
Results
We included 138 subjects, with left- (n = 48; 21 men and 27 women; mean age ± SD: 57.3 ± 12.3 years) and right-sided (n = 44; 19 men and 25 women; mean age ± SD: 59.0 ± 13.1 years) surgically naive AN patients, and NH healthy controls (n = 46; 24 men and 22 women; mean age ± SD: 54.0 ± 11.7 years). PTA scores prior to surgery were collected for 75/92 AN patients, and were significantly poorer at the ipsilateral ear compared to the contralateral ear in this group ($p < 0.05$). Whole-brain fixel-based analysis accounting for age and sex between either right- or left-sided AN patients and NH healthy controls showed increased FD, FC, and FDC ($p < 0.05$) within non-auditory cortical and brainstem tracts for the AN patients. There were no relationships between duration or severity of hearing loss with the combined fibre density and cross-section metric at each significant tract identified with the fixel-based analysis in the AN group.
Conclusion
We found significant white matter micro- and macrostructural increases in the AN group across non-auditory tracts from the brainstem to the cortex. Our findings suggest whole-brain abnormalities in AN patients with one-sided hearing loss, which may comprise a target list of regions important for auditory therapies and neuro-prosthetic design. Future analysis will assess whether auditory tract structure is linked to changes in the non-auditory tracts and to the degree of hearing loss in this patient group.

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Poster

P481. Visual Motion II

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Program #/Poster #: P481.01

Topic: D.07. Vision

Title: Attentional load modulates bottom-up processing of biological motion in the occipito-temporal cortex

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Abstract: Humans can detect biological motion (BM) even when the stimulus is a point-light display of an actor. The gender, emotional state, and social intentions of the performer can be distinguished easily. Thus, it can be said that BM perception occurs effortlessly in an automated manner. Thornton and Vuong (2004) showed that task-irrelevant BM in the periphery interfered with task performance at the fovea. However, the neural underpinnings of the bottom-up processing of BM lacks thorough examination in the field. Under selective attention, BM perception is supported by a network of regions in the occipito-temporal cortex including motion and form sensitive areas and superior temporal sulcus, in addition to the frontal regions such as premotor cortex (PMC) and inferior frontal gyrus (IFG). A retinotopy study by Saygin and Sereno (2008) showed that task-irrelevant BM stimuli recruit retinotopic maps in occipito-temporal cortex. Based on this finding, we investigated how bottom-up perception of BM would be processed under attentional load. Participants (N=25) underwent a functional magnetic resonance imaging (fMRI) study. They performed a visual detection task at the fovea while attentional load was manipulated (high vs low load). At the same time, task-irrelevant BM stimulus was shown in the periphery. The univariate analysis and MVPA results showed that the bottom-up processing of BM was associated with the motion-sensitive areas in the occipito-temporal cortex. Furthermore, during the low attentional load condition, BM related activation was found stronger compared to that of in high attentional load condition. Thus, it can be concluded that BM is processed even when it is away from the focus of attention, and this processing is modulated by attentional load.

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Poster

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Support: NIH Grant EY027373-02

Title: Persistent representation of image speed in visual area MT even during poor steady-state smooth pursuit eye movements

Authors: *S. C. BEHLING, S. G. LISBERGER;
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Abstract: Primates use smooth pursuit eye movements to track moving objects. Smooth pursuit is initiated by sensory estimates of stimulus speed represented in the middle temporal (MT) area of extrastriate visual cortex. After initiation, steady-state tracking can be almost perfect and, because perfect tracking eliminates image motion, it must be supported by extra-retinal motor feedback to maintain eye speed at target speed. However, situations exist where steady-state eye speed is persistently less than target speed, yet the tracking error goes uncorrected. For example, we showed previously that reducing the coherence in a patch of dots that a monkey is tracking degrades the eye speed during both the initiation and steady-state of pursuit. Steady-state eye speed lagged target speed persistently even in the presence of image motion. To understand the representation of image motion during steady-state tracking and to ask why persistent image motion is not corrected by the pursuit system, we recorded from isolated single neurons in extrastriate visual area MT. We sought to determine whether steady-state eye speed lags behind target speed for low dot coherence because (i) the speed representation in MT is reduced or (ii) the representation of image speed remains accurate in MT and eye speed is eroded further downstream within the motor feedback loop. We presented moving patches of dots of varying speeds and coherences while recording neurons in area MT. During pursuit initiation, the amplitude, but not the tuning, of MT responses depends on dot coherence. Comparison of neural responses during pursuit initiation and steady-state tracking shows that activity during initiation is largely able to predict activity throughout pursuit based solely on the motion of the stimulus relative to the eye. We conclude that MT consistently represents speed across the full pursuit response for all dot coherences. Visual motion may not have a strong impact on steady-state eye speed because the output from MT is lower in amplitude and is interpreted as having lower signal-to-noise even though the signal itself is not disrupted. Or, the extraretinal signals that normally sustain steady-state eye speed may be compromised by low dot coherences. Further exploration of neural responses in downstream parts of the pursuit circuit will be needed to fully understand why the pursuit system fails to correct persistent visual motion during steady-state tracking.

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Poster

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Topic: D.07. Vision

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HMWK TAM

Title: Neural processing of perturbed self-motion in macaque area VIP

Authors: *J. C. B. SCHWENK[1,2], J. CHURAN[1,2], D. ENGEL[1,2], A. KAMINIARZ[1,2], F. BREMMER[1,2];
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Abstract: The visual perception of self-motion is fundamental to our navigation through an environment. The neural processing of self-motion signals is often studied using optic flow patterns that simulate a smooth and continuous movement. However, the optic flow available naturally, e.g. during walking, is less regular, owing to movements of the observer or often unrelated movements in the environment. Here, we investigated how perturbations impact neural responses in the ventral intraparietal area (VIP), which shows strong representations of visually simulated self-motion. Previous studies have demonstrated predictive activity in VIP associated with expected motion onset (Churan et al., JNP, 2021). Accordingly, we were also interested whether the predictability of a perturbation would modulate its impact on ongoing activity.
We performed extracellular recordings from single neurons in area VIP of one macaque monkey. Stimulation consisted of simulated self-motion through a 3D cloud of random dots along one of two orthogonal axes in the horizontal plane. On every trial, the velocity of the self-motion followed a sinusoidal profile, oscillating at 1 cycle/second between opposite movement directions. This regular pattern was perturbed by a brief (40 ms) jerk of linear motion along the axis orthogonal to the main self-motion, at varying time points in the trial. Between different experimental blocks, the onset of the perturbation was either predictable or random.
Self-motion stimuli induced strong responses, characterized by ON (movement in the preferred direction) and OFF (non-preferred direction) states. Perturbations induced transient increases in activity. These were subadditive to the ongoing self-motion response, with largest incremental activity during a neuron's OFF-state. Early and late phases of the self-motion were differentially affected by the predictability of a perturbation. The transient response to self-motion-onset was unselectively suppressed within blocks including a perturbation, independent of its expected onset. In contrast, responses to predictable perturbations during the oscillatory steady-state were selectively attenuated.
These findings provide further evidence for a predictive role of area VIP in the processing of visual self-motion. The suppression of activity associated with the expectation of a perturbation is in line with the theoretical framework of predictive coding. Our results also confirm previous

observations that VIP neurons signal the onsets of visual events independently of their tuning for self-motion. This supports a more general role of area VIP in the monitoring of self-motion through space.

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Poster

P481. Visual Motion II

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Topic: D.07. Vision

Support: NIH EY07556 & EY13312

Title: Non-rigidities from motion-energy are turned rigid by tracking salient features

Authors: *A. MARUYA, Q. ZAIDI;
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Abstract: How does the brain make objects appear rigid across multiple views when retinal images are deformed non-rigidly by observer or object motion, especially for objects where motion parallax and correspondence between perspective views are not sufficiently informative? To get an answer, we used perceptual experiments and neural modeling on rotating rigid objects that can appear rigid or non-rigid depending on speed, to test whether it is tracking of salient features that counteracts nonrigidity. When two 3-D circular rings are rigidly linked at an angle and rotated around an axis oblique to both at a moderate speed, they appear as if they're rolling on each other and wobbling. This illusion of a non-rigid connection is striking enough to have been used in shop windows and in movies. Using a model of arrays of V1 direction selective cells, we show that despite the object being physically rigid, because of the oblique axis rotation, the pre-dominant motion energy at the contours of the rings supports a percept of the rings wobbling non-rigidly against one another instead of rotating rigidly. There are no physical cues that could support the illusion of rolling in the pair of circular rings, suggesting that this illusion is generated purely by the brain to make the wobbling appear physically feasible. That contention is supported by the presence of the rolling illusion when the rings are physically wobbled but not rolled. By asking observers to make forced-choice judgments between the link being rigidly connected or not, we quantified that the circular rings appear non-rigid at moderate speeds (6.0 deg/sec) but rigidly connected at slow speeds (0.6 deg/sec). However, the rings appear rigid at 6.0 deg/sec if the link is painted or replaced by a gap, or the rings are octagons with prominent vertices. Phenomenologically, the motion of painted segments, gaps, or vertices provides cues for rotation and against rolling, which in turn eliminates the now unfeasible

illusion of wobble. A model of arrays of MT pattern-motion cells tracks the rotational motion of the vertices of polygonal rings, and thus contributes to a percept of rigid rotation. However, at high speeds (60 deg/sec), all of these configurations appear non-rigid. Salient features thus contribute to rigidity at slow and moderate speeds, but not at high speeds. An image-based model being obviously insufficient, a generative model that combines motion-energy signals from the contours, with signals from MT pattern cells tracking salient features, explains the rigidity and non-rigidity of the rings and other rotating objects, by incorporating a minimum speed threshold for motion energy and a maximum speed cut-off on feature tracking.

Disclosures: A. Maruya: None. **Q. Zaidi:** None.

Poster

P481. Visual Motion II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P481.05

Topic: D.07. Vision

Support: NIH grant R01 EY022443
McPherson Eye Research Institute Kenzi Valentyn Vision Research Award
Wisconsin Alumni Research Foundation

Title: Neural encoding of multiple moving surfaces and choice-related activity in cortical area MT - role of disparity in image segmentation

Authors: *A. CHAKRALA, J. XIAO, X. HUANG;
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Abstract: Motion and binocular disparity provide crucial cues for image segmentation. We investigated the role of MT, a cortical area important for motion and disparity processing, in segmenting multiple surfaces. We recorded from neurons in two macaque monkeys as they performed a task requiring segmentation. The visual stimuli were overlapping random-dot patches moving in two directions separated by 60° or 120°. One patch was presented at a near disparity and the other at a far disparity. We varied the vector-average direction of the two patches to measure response tuning to the bi-directional stimuli and presented a stationary disparity cue before each trial. The animals were trained to report the direction at the cued disparity. The disparity preference of each neuron was determined by the response to a single patch. Animal B performed equally well at discriminating the direction of near or far patch of the overlapping stimuli, whereas animal G performed significantly better at discriminating the near patch direction. We found that, for animal B, the direction tuning of a neuron to two patches was significantly biased toward the patch located at a more preferred disparity of the neuron, more than expected by response averaging. In contrast, MT neurons in animal G showed a significant

bias toward the near patch, regardless of the disparity preference of a neuron or the cued disparity. Nevertheless, the bias toward the near patch was significantly stronger for the near- than the far-preferred neurons. We recorded from a third animal while the attention was directed away from the receptive fields. We found the same bias toward the patch at a more preferred disparity, as in animal B. Interestingly, MT neurons showed choice-related activity that depended on the disparity of the target patch, the disparity preference of the neuron, and the behavioral/neuronal bias. For animal B, choice probability calculated based on the responses of neuronal populations suggests that the activity of near-preferred neurons is linked to the choice of the near direction of overlapping stimuli, whereas the activity of far-preferred neurons is linked to the choice of far direction. For animal G that showed a significant bias toward the near patch, activity of both near- and far-preferred neurons is linked to the choice of near direction. Our results suggest that MT neurons encode multiple stimuli by preferentially representing the stimulus located at a more preferred disparity of a given neuron. The disparity tuning and choice-related activity in MT allow individual components of multiple stimuli to be represented by different groups of neurons and therefore provide a population code that aids segmentation.

Disclosures: A. Chakrala: Other; Neurosciene Training Program. **J. Xiao:** Other; Physiology Graduate Training Program. **X. Huang:** Other; McPherson Eye Research Institute.

Poster

P481. Visual Motion II

Location: Virtual Only

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Program #/Poster #: P481.06

Topic: D.07. Vision

Support: NIH Grant R01 EY022443
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Human Frontier Science Program (HFSP) Young Investigator Grant RGY0079/2018

Title: Role of MT disparity tuning biases in figure-ground segregation

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[1]Sch. of Optometry, Univ. of California, Berkeley, Berkeley, CA; [2]Brain Cognitive Sci., Univ. of Rochester, Rochester, NY; [3]Dept. of Neurosci., Univ. of Wisconsin Madison, Madison, WI

Abstract: Segregating salient figures in visual scenes from the surrounding context is a fundamental process in spatial perception, often referred to as figure-ground segregation. While several visual cues exist to support this process, binocular disparity and motion have been identified as key cues in primate vision. Figure-ground segregation likely depends on multiple

cortical areas, but area MT appears uniquely suited for supporting this task given its tuning for disparity and motion. Previous work investigating natural scene statistics found that multiple surfaces with different disparities are often present within visual field regions of similar size to MT receptive fields (RFs). MT neurons have also been shown to nonlinearly modulate their responses when multiple surfaces with different binocular disparities fall within their RFs. Given these findings, we hypothesized that the neuronal population in MT represents disparity information in a way that facilitates efficient figure-ground segregation. To address this hypothesis, we created model MT populations with biased or unbiased tuning characteristics. For example, neurons in MT more frequently exhibit odd-symmetric disparity tuning curves than neurons in V1 and they also generally prefer crossed disparities over uncrossed disparities. We used a database of stereoscopic natural images as input to the model MT population and used laser range measurements registered to each image to recover the ground truth disparities in each model neuron's RF. The figure-ground boundaries in each image were previously labeled by human participants, which we used to separately calculate the distribution of disparities in figure and ground regions. We find that MT-like biases in disparity tuning improve the mutual information between the scene disparities at each RF location and the MT responses. Next, we compared the distribution of disparities found in figure regions of the images with those in the ground regions, and formulated their discimination as a signal detection problem. The results suggest that the MT disparity tuning biases can increase the discriminability of figure regions by allocating more neural resources to the disparities surrounding the criterion value that maximizes correct classification. Thus, the representation of disparity in MT may support figure-ground segregation both by exploiting the first-order disparity statistics present in natural scenes and by maximizing sensitivity to the most informative disparities for figure-ground segmentation.

Disclosures: T.S. Manning: None. **E. Alexander:** None. **G.C. DeAngelis:** None. **X. Huang:** None. **E.A. Cooper:** None.

Poster

P481. Visual Motion II

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Topic: D.07. Vision

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5 R90 DA 033461-08
EY027023

Title: A single neuron correlate for long-range apparent motion in the primate brain

Authors: *A. BIGELOW[1], T. NAMIMA[2], T. KIM[2], W. BAIR[2], A. K. PASUPATHY[2];
[2]Biol. Structure, [1]Univ. of Washington, Seattle, WA

Abstract: When a stimulus saltates across the visual field with relatively large spatial displacements (dX) and slow temporal steps (dT), it induces a strong motion percept known as long-range apparent motion. Despite decades of study, no neuronal correlate to has been identified along the primate dorsal visual stream, the quintessential motion processing pathway. This may be because, as psychologists have long argued, the percept of long-range motion relies on object-based, higher-order tracking processes along the ventral visual stream. To test this hypothesis, we studied responses of individual neurons in primate cortical area V4, a fundamental, mid-level ventral stream area for object form processing that has strong interconnections with key motion processing areas along the dorsal stream, such as areas MT and LIP. We measured the responses of 115 neurons in two monkeys to noise and sinusoidal patches that moved across the visual display with spatial and temporal displacements that satisfied the constraints of long-range motion (dX > 0.5 deg, dT = 100 ms) in 8 directions at 45° increments. Using a standard direction index (DI), we found that 30% neurons in V4 (34/115) are direction selective for long-range motion (DI $\geq$ 0.5). Because the mean luminance of the noise patches were equal to that of the background, this motion direction selectivity may be based on second-order cues. We also found that V4 direction selectivity was position invariant and preserved across a range of spatial and temporal displacements that spanned short- and long-range regimes. Surprisingly, however, we find that direction selectivity in many neurons disappears in response to local drift, i.e., motion behind an aperture, raising the intriguing prospect that the direction selectivity we observed may be specific to the movement of object boundaries. To explicitly test this hypothesis, we measured neuronal responses to sinusoidal patches that either drift behind a static aperture or jump across the visual display. At identical speeds, neurons exhibit direction selectivity to a stimulus that translates across the visual field but not to one that drifts, further supporting the notion of a higher-order, object-based motion detection system in V4 that differs substantially from models that explain direction selectivity in V1 and MT. Finally, we find compelling evidence that V4 direction selectivity can be driven by higher-order (e.g., 3rd-order) cues, such as chromatic boundaries. These results support a complementary motion processing system in the ventral stream that may be critical for tracking object motion in occluded environments where information may be intermittently available.

Disclosures: A. Bigelow: None. **T. Namima:** None. **T. Kim:** None. **W. Bair:** None. **A.K. Pasupathy:** None.

Poster

P510. Cerebellum- Long Range Connections I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P510.01

Topic: E.02. Cerebellum

Support: NIH 4R00NS097620
AHA 847486
CNSM D000000101
NSF 2048231

Title: Coordinated cortico-cerebellar neural dynamics underlying neuroprosthetic learning

Authors: *A. ABBASI[1], N. DANIELSEN[1], T. GULATI[1,2];
[1]Cedars-Sinai Med. Ctr., Los Angeles, CA; [2]Bioengineering and Med., Univ. of California Los Angeles, Los Angeles, CA

Abstract: Introduction: Brain-machine interfaces (BMIs) or neuroprosthetics allow neural control over assistive devices. They also provide an important framework for studying neural plasticity. Recent studies have suggested that optimal engagement of learning is essential for robust neuroprosthetic control. However, little is known about the neural processes in subcortical regions that support learning a neuroprosthetic skill. We performed electrophysiologic recordings in the motor cortex (M1) and the cerebellum (Cb) of rodents while they engaged M1 neurons in BMI control. By monitoring neural ensembles in both regions, we found robust indirect modulation of neurons and emergence of coordinated activity in the local field potentials (LFPs) of M1 and Cb. **Methods:** We have recorded single units and LFPs in adult Long-Evans rats (n=6) while they performed a neuroprosthetic task by implanting microwire arrays (Tucker-Davis Technologies, FL) in M1 and silicon tetrodes (Neuronexus, MI) or drivable polytrodes (Cambridge Neurotech, UK) in Cb. During the task, activity of a subset of M1 neurons was transformed into the angular velocity of a feeding tube using a simple linear decoder. Rats modulated the activity of these 'direct' neurons to obtain a water reward. We analyzed how the activity in these direct neurons, as well as all other recorded (indirect) neurons in M1 and Cb changed while learning the neuroprosthetic task. We also analyzed band-limited oscillatory activity in both regions. Furthermore, we performed optogenetic silencing of Cb by injecting red-shifted halorhodopsin (Jaws), while rats (n=2) performed the neuroprosthetic task and analyzed how cerebellar silencing affected the M1 activity and neuroprosthetic learning. **Results:** We found that learning successful BMI control was associated with robust modulation of 'direct' neurons in M1, as well as robust 'indirect' neuron's modulation in M1 and Cb. Furthermore, we observed the emergence of task-related 3-6 Hz synchronous oscillatory activity in cortico-cerebellar LFPs which also modulated the task-related neural spiking. We found that these trends emerged late in learning once skillful BMI control was exerted. Additionally, we found that correlations between rat forelimb movement and the movement of the feeding pipe reduced with BMI learning. **Conclusion:** Our work has identified neural mechanisms in M1 and Cb which are associated with learning of a cortically-controlled neuroprosthetic task. This underscores the importance of optimal engagement of neural learning mechanisms in an offsite motor region- the cerebellum, for successful learning of M1-controlled neuroprosthetic task.

Disclosures: A. Abbasi: None. **N. Danielsen:** None. **T. Gulati:** None.

Poster

P510. Cerebellum- Long Range Connections I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P510.02

Topic: E.02. Cerebellum

Support: NIH 4R00NS097620
NSF 2048231
AHA 847486

Title: Low-frequency neural dynamics emerge across cortico-cerebellar networks with motor skill learning

Authors: P. FLEISCHER[1], A. ABBASI[1], N. DANIELSEN[1], *T. GULATI[1,2];
[1]Ctr. for Neural Sci. and Med., Cedars-Sinai Med. Ctr., Los Angeles, CA; [2]Bioengineering and Med., Univ. of California Los Angeles, Los Angeles, CA

Abstract: *Introduction*: Performing skilled actions that involve reaching, grasping and manipulating objects in our environment is a defining ability of mammals with prehensile forelimbs. Such skillful movement control requires coordination between several brain areas that are reciprocally connected through mono- or polysynaptic pathways. We have performed extracellular electrophysiologic recordings from the motor cortex (M1) and the cerebellum (CB) while rats learn a skilled reach-to-grasp (RTG) task and report the emergence of low-frequency oscillatory (LFO, 1.5-4Hz) activity in these regions with learning this skill. *Methods*: Single-unit and local field-potential (LFP) recordings were collected from the M1 and CB's Simplex, Crus I, and Crus II lobules while the rats (n=4) were trained in the RTG task for five days. We analyzed the behavior changes from early days of training to late days and the associated power-spectral changes in M1 and CB. We also analyzed movement-related LFP power and neural synchrony across the M1 and CB. Finally, we explored the representation of single neuronal activity in M1 and CB during learning by evaluating sequential activity of neurons in relation to movement onset. *Results*: During movement we observed coordinated low-frequency activity across the M1 and CB that was closely related to the reach onset in the RTG task. Movement-related LFO power in M1 and CB LFPs increased from day 1 to day 5 by ~59% and ~63%, respectively. Furthermore, we also found that this LFO activity in LFPs modulated the phasic spiking activity of the neurons in each region. Single neuronal activity sequences showed stronger modulation around the start of the reaching motion. *Conclusions*: Our work shows that coordinated neural activity emerges across M1 and CB with the dexterous skills associated with RTG task learning. Understanding this phenomenon has implications for neurological disorders such as cortical stroke or cerebellar disorders where dexterous motor control is impaired. Our next goal is to develop a neural network model of the changing neural dynamics over the course of learning.

Disclosures: P. Fleischer: None. **A. Abbasi:** None. **N. Danielsen:** None. **T. Gulati:** None.

Poster

P510. Cerebellum- Long Range Connections I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P510.03

Topic: E.02. Cerebellum

Support: Pew Scholars Program
NIH NS112312
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Simons Collaboration on the Global Brain

Title: An activity map of the cerebellum underlying motor planning

Authors: *J. ZHU[1], H. HASANBEGOVIC[2], Z. GAO[2], N. LI[1];
[1]Dept. of Neurosci., Baylor Col. of Med., Houston, TX; [2]Dept. of Neurosci., Erasmus MC, Rotterdam, Netherlands

Abstract: The neocortex and the cerebellum are thought to interact during cognitive functions. The two brain regions are reciprocally linked through the pons and the thalamus to form cortico-cerebellar loops. However, the anatomical and functional organization of cortico-cerebellar loops underlying specific behavior remain unclear. During motor planning, a localized region of the mouse frontal cortex (anterior lateral motor cortex, ALM) exhibits preparatory activity instructing upcoming movements. Recent evidence shows that preparatory activity in ALM is dependent on the cerebellum (Gao et al., Nature 2018; Chabrol et al, Neuron 2018). ALM and the fastigial nucleus (FN) are parts of a cortico-cerebellar loop that mediates motor planning of directional licking. Here we mapped cortico-cerebellar input-output connectivity in the cerebellar cortex and present the first activity map of the cerebellar cortex supporting motor planning. Using trans-neuronal anterograde viral tracing, we mapped ALM inputs to the cerebellar cortex via the mossy fibers. Labeled mossy fiber boutons were comprehensively imaged and annotated across the cerebellar cortex. Using monosynaptic rabies tracing, we labeled the Purkinje cells targeting thalamus-projecting fastigial nucleus neurons, which provide the output back to ALM. Complementing these anatomical connectivity maps, we densely surveyed activity across the cerebellar cortex using silicon probe recordings during a delayed response task in which mice used short-term memory to plan directional licking. Anatomy and electrophysiology data were aligned into the Allen Mouse Common Coordinate Framework, facilitating comparisons of the connectivity and activity maps. ALM provided diffused inputs to the cerebellar cortex and large swaths of the cerebellar cortex sent output back to ALM. Yet only a localized region (spanning crus 1 & 2) with conjunction of input-output connectivity with ALM was selectively enriched in preparatory activity. A second region in the posterior vermis (lobule 7-9) with only output

connectivity to ALM also exhibited preparatory activity. Optogenetic perturbation of Purkinje cells in these regions, but not other regions, during motor planning biased subsequent licking direction. These results identified two regions of the cerebellum that formed functional connections with ALM to mediate motor planning and suggest that precise reciprocal connections between localized regions of frontal cortex and cerebellum mediate specific cognitive functions.

Disclosures: J. Zhu: None. **H. Hasanbegovic:** None. **Z. Gao:** None. **N. Li:** None.

Poster

P510. Cerebellum- Long Range Connections I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P510.04

Topic: E.02. Cerebellum

Support: Human Brain Project SGA3 Grant 945539

Title: Integration of cerebro-cerebellar loops improves virtual brain models of neurodegenerative diseases

Authors: A. MONTEVERDI[1,2], ***F. PALESI**[2], C. GANDINI WHEELER-KINGSHOTT[3,2,1], E. U. D'ANGELO[2,1];
[1]Brain Connectivity Ctr., IRCCS Mondino Fndn., Pavia, Italy; [2]Dept. of Brain and Behavioral Sci., Univ. of Pavia, Pavia, Italy; [3]NMR Res. Unit, Queen Square MS Centre, Dept. of Neuroinflam., UCL Inst. of Neurol., London, United Kingdom

Abstract: The Virtual Brain (TVB) is a novel neuroinformatic platform allowing to simulate whole-brain dynamics starting from individual structural (SC) and functional connectivity (FC). To date, TVB has been used to characterize brain dynamics both in healthy and pathological subjects but cerebellar nodes and their connections have been overlooked. This is at odd with the recent observation that integrating cerebro-cerebellar connections can improve TVB predictive capability in healthy subjects. This raises an issue because cerebellar impairment has been reported in neurodegenerative diseases, like Alzheimer's disease (AD), Frontotemporal Dementia (FTD) and Amyotrophic Lateral Sclerosis (ALS). In this work we integrated cerebro-cerebellar connections in TVB to assess the cerebellar impact on simulated brain dynamics both in physiological conditions (healthy controls) and neurodegenerative states (AD, FTD, ALS). Simulations were performed using three different combinations of connections and nodes: (i) whole-brain nodes and connections, (ii) cerebral cortex nodes and connections only, (iii) cerebral cortex nodes considering cerebro-cerebellar connections ("embedded cerebro-cerebellar subnetwork"). The Wong-Wang model was chosen to gain information on global brain dynamics and local excitatory/inhibitory balance. Pearson Correlation Coefficient (PCC) was computed

between experimental FC and simulated FC for all groups and networks. Within each pathological group, the PCC values obtained with the embedded cerebro-cerebellar subnetwork were significantly higher than those obtained otherwise. These findings demonstrate that the integration of cerebro-cerebellar connections in TVB improves the predictive power of the model in pathological conditions, supporting the cerebellar involvement in neurodegenerative states. In addition, the biophysical parameters derived from TVB were different between the clinical phenotypes considered. In backward regressions, TVB parameters significantly contributed to explain the variation of neuropsychological scores in physiological and pathological conditions. Moreover, cluster analysis provided a unique description of the excitatory/inhibitory balance at the single subject level. In summary, this work highlights the cerebellar involvement in neurodegenerative states, opens new perspectives in the use of TVB to explore neurodegenerative mechanisms and lays the basis for future personalized diagnosis and therapy.

Disclosures: A. Monteverdi: None. **F. Palesi:** None. **C. Gandini Wheeler-Kingshott:** None. **E.U. D'Angelo:** None.

Poster

P510. Cerebellum- Long Range Connections I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P510.05

Topic: E.02. Cerebellum

Support: Seed funds to BW from Rowan University

Title: Organization of the locus coeruleus-norepinephrine projection to the rodent cerebellum.

Authors: *B. WATERHOUSE[1], H. PREDALE[1], D. J. CHANDLER[1], N. PLUMMER[2], P. JENSEN[2];
[1]Cell Biol. and Neurosci., Rowan Univ. Sch. of Osteo. Med., Stratford, NJ; [2]Lab. of Neurobio., NIEHS/NIH, Research Triangle Park, NC

Abstract: The brainstem nucleus locus coeruleus (LC) projects widely throughout the forebrain, brainstem, cerebellum, and spinal cord and is a major source of the small molecule transmitter norepinephrine (NE) in these areas. While we know much about the organization of the LC-NE system with respect to sensory and cognitive circuitries and the impact of LC output on sensory guided behaviors and executive function, much less is known about the role of the LC-NE pathway in motor network operations and movement control. As a starting point for closing this gap in understanding, we are using the intersectional recombinase-based viral-genetic strategy TrAC (Tracing Axon Collaterals) to characterize LC-NE projections to sub-regions of the rodent cerebellum, a structure that is prominently engaged in maintaining posture, and planning and executing movement. This technique allows us to not only map the distribution of LC-NE axons

to target regions of the cerebellum, but also to examine the distribution of their cell bodies within the LC and their dendritic fields within the peri-coerulear space. Initial results indicate the fastigial and interposed nuclei of the cerebellum receive input from a large number of NE-containing neurons distributed with an ipsilateral bias; 55-65% of total labeling in the ipsilateral LC and 35-45% of the total labeling in the contralateral LC. LC cells that project to cerebellum are displaced dorsally throughout the rostral-caudal extent of the nucleus. This pattern contrasts sharply with the scattered and predominantly ipsilateral (95%) distribution of the NE-containing cells that project from LC to cerebral cortex including the primary motor cortex. The dendritic fields of cerebellar-projecting LC neurons are concentrated laterally and medially within the peri-coerulear space. Overall, these studies begin to provide a detailed map and characterization of LC noradrenergic projections to sub-regions of the cerebellum. The initial results reveal substantial differences in the organization of the LC-NE projection to cerebral cortex vs cerebellum and identifies the regions of the peri-coerulear space where inputs to LC-cerebellar projecting cells can occur.

Disclosures: B. Waterhouse: None. **H. Predale:** None. **D.J. Chandler:** None. **N. Plummer:** None. **P. Jensen:** None.

Poster

P510. Cerebellum- Long Range Connections I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P510.06

Topic: E.02. Cerebellum

Support: NIH R01 NS045193
NIH R01 MH115750
Fellowship by New Jersey Autism Center of Excellence (CAUT20AFP006)
Netherlands Organization for Scientific Research—Veni ZonMW, 91618112
Erasmus MC Fellowship 106958

Title: The role of locus coeruleus-driven neuromodulation in the deep cerebellar nuclei for associative learning

Authors: *J. LEE, H.-J. BOELE, S. S.-H. WANG;
Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ

Abstract: The locus coeruleus (LC) modulates the activity of many brain regions, including the cerebellar nuclei. LC may play a modulatory role during cerebellum-dependent learning based on lesion, pharmacological, and microdialysis evidence. However, these methods lack temporal precision. Here we investigated the relationship between endogenous patterns of noradrenergic signaling in delay eyeblink conditioning, both over the course of learning and during trial-by-

trial performance after learning has taken place. First, we confirmed that LC indeed innervates cerebellar nuclei, including the interpositus, which supports eyeblink conditioning. Second, we showed that photostimulation of ChR2-expressing LC terminals in cerebellar nuclei releases norepinephrine using fast-scan cyclic voltammetry. Third, we performed eyeblink conditioning experiments in adult mice while simultaneously measuring activity of axonal GCaMP8f-expressing LC axons in the cerebellar nuclei and jRGECO1a-expressing cerebellar nuclei using fiber photometry. For eyeblink conditioning, a blue-LED conditional stimulus (CS) was repeatedly paired with an unconditional stimulus (US) corneal airpuff, which led to the acquisition of conditioned eyeblink responses in response to the CS. After learning, we analyzed trial-by-trial correlation of LC activity and the probability of conditioned eyelid responses. We found an inverse correlation between pre-CS LC activity and the probability of a conditional response. In addition, we found an inverted-U-shaped relationship between during-CS GCaMP8f signal in LC axons and the conditioned response probability. Fourth, we found that photostimulation of ChR2-expressing LC terminals in cerebellar nuclei impairs motor learning. Our findings suggest an inverse or inverse-U-shaped relationship between norepinephrine levels in the cerebellar nuclei and both learning and expression of conditioned motor responses, consistent with proposed linkages between noradrenaline, arousal, and learning (Aston-Jones & Cohen, Annu. Rev. Neurosci., 2005).

Disclosures: J. Lee: None. **H. Boele:** None. **S.S. Wang:** None.

Poster

P510. Cerebellum- Long Range Connections I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P510.07

Topic: E.02. Cerebellum

Support: NIH Grant R01 MH115604 03
NIH Grant R01 DA044761-03

Title: Cerebellar modulation of the hypothalamus: a potential role in regulation of survival behaviors?

Authors: *N. S. J. CAYLA[1], M. G. OÑATE[1], J. A. WILLETT[3], J. A. VERA[2], K. KHODAKHAH[4];
[2]Neurosci., [1]Albert Einstein Col. of Med., Bronx, NY; [3]Biol. Sci., North Carolina State Univ., Raleigh, NC; [4]Dept Neurosci., Albert Einstein Col. Med., Bronx, NY

Abstract: Although it constitutes a mere 10% of the total volume of the human central nervous system, the cerebellum contains more than half of our neurons. It is well-known for its involvement in motor control and coordination, but recent observations made on patients

suffering from a myriad of mental disorders, or those with cerebellar lesions have led to a growing appreciation for its roles in the regulation of cognitive and somatic functions as well. To delineate the non-motor functions of the cerebellum it is important to have a detailed map of its connectivity with other brain regions. So, we used anatomical neuronal tracings to explore the potential presence of monosynaptic projections from the deep cerebellar nuclei (DCN) to the hypothalamus. To do so, we injected an AAV1-cre virus in the DCN of a reporter mice (RCE:LoxP), in which neurons require Cre to express GFP. This virus possesses transsynaptic spread properties and allows for the labelling of the somas of monosynaptic target-cells. With this approach, DCN target-cells in several hypothalamic regions were identified: in the Medium Pre-Optic Area (MPOA), in the Lateral Hypothalamic Area (LHA), and in the Paraventricular Nucleus of the Hypothalamus (PVH). In the PVN immunostaining revealed that oxytocin- and vasopressin-expressing neurons receive direct projections from the DCN. Functionally, using in vivo electrophysiology, we found that optogenetic activation of cerebellar axons in the Paraventricular Nucleus of the Hypothalamus increased the firing rate of the target neurons. In addition, we found that optogenetic activation of DCN projections in the hypothalamus increased cFos expression in the PVN. Optogenetic activation of cerebellar axons in the PVN did not produce any overt motor dysfunction. These results suggest that the cerebellum may have a modulatory role in the hypothalamus and may be involved in PVN-regulated behaviors such as social behaviors, and stress or circadian regulation among others. In future experiments, I plan to characterize this novel brain circuit by combining cell-type specific neuroanatomical tracing with in *vivo* and *in vitro* electrophysiological assays in behavioral tasks.

Disclosures: N.S.J. Cayla: None. **M.G. Oñate:** None. **J.A. Willett:** None. **J.A. Vera:** None. **K. Khodakhah:** None.

Poster

P598. Stress and Neuroimmunology

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P598.01

Topic: F.04. Stress and the Brain

Support: NIH Grant R01 NS094306-01A1
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The Robert E. and Donna Landreth Fund for the Study of Neuroimmune Interactions in Autism

Title: Brain inflammation induced by COVID-19 related disruptions in non-infected healthy individuals, as revealed by multimodal imaging

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Abstract: Beyond the staggering number of infections and deaths, the COVID-19 pandemic has caused lifestyle, societal, and other disruptions, impacting our lives in multiple ways. Following the beginning of the March 2020 lockdown, even non-infected and otherwise healthy individuals have begun commonly reporting fatigue, depression, “brain fog” and other “sickness behaviors”. As these may be behavioral manifestations of neuroimmune activation, we tested the hypothesis that the COVID-19 pandemic-related disruptions may have caused neuroinflammation. To this end, we conducted a multimodal, retrospective evaluation of Positron Emission Tomography / Magnetic Resonance (PET/MR) data collected in healthy subjects pre- and post- lockdown in our Center. 57 ‘pre-lockdown’ subjects (scan dates: 04/2012-02/2020) were compared against 13 ‘post-lockdown’ subjects (08/2020-04/2021), negative to COVID-19 PCR antibody test. All subjects were scanned with a PET/MR tomograph and [11C]PBR28, which binds to the glial marker TSPO. A subset (13 pre-lockdown; 10 post-lockdown) were also scanned with Magnetic Resonance Spectroscopy (MRS). Pre- and post-lockdown [11C]PBR28 standardized uptake value ratios were compared in both region-of-interest analyses in areas previously associated with stress or depression (accumbens, hippocampus, thalamus, anterior cingulate cortex) and in voxel-wise analyses (FSL randomise; cluster-forming threshold of p=0.01; cluster size threshold of p = 0.05). Concentrations of the glial marker myo-inositol (mIns) and, for reference, Creatine (Cr; a cellular energetic marker) and N-Acetyl Aspartate (NAA; a marker of neuronal integrity) were extracted from an 15mm isotropic voxel placed in the left thalamus. Elevations in both PET and MRS neuroinflammatory markers were seen in the ‘post-lockdown’ cohort. Significant post-lockdown [^{11}C]PBR28 uptake elevations were observed in all the a-priori ROIs ($0.005<p<0.035$), as well as in the voxel-wise group contrast (sensory, motor and higher order association areas, as well as white matter). MRS data also revealed significantly higher concentrations of mIns in the post-lockdown cohort (p=0.005), whilst no statistically significant differences in Cr and NAA were observed across groups (p>0.20), as expected. Our results suggest that COVID-19 related disruptions may have caused neuroinflammation, though the clinical significance of our observations awaits further investigation. Elevated neuroimmune responses may help explaining the pandemic-related "sickness behaviors", which are symptoms commonly reported also among individuals that were never infected by the virus itself.

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Poster

P598. Stress and Neuroimmunology

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Program #/Poster #: P598.02

Topic: F.04. Stress and the Brain

Support: Department of Veterans Affairs Grant #I21BX002664
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Title: Progressive dysregulation of the cholinergic anti-inflammatory network in a rodent model of Gulf War Illness

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Abstract: Soldiers who served in the 1990-1991 Gulf War returned with an unexplained, multi-symptom illness that continues to affect over 250,000 veterans. This constellation of symptoms, which includes cognitive disturbances, immune dysregulation, and cardiovascular abnormalities, is now termed the Gulf War Illness (GWI). The cause of this unique symptomatology is still unknown; however, researchers have identified the prophylactic use of the acetylcholinesterase inhibitor, pyridostigmine bromide (PB), and the stress of combat as two potential contributing factors. GWI is especially problematic as the symptoms have become progressively worse in affected individuals, while long-term, pre-clinical studies evaluating this progression remain limited. As inflammation has been suggested as a mechanistic mediator of the progressive nature of GWI pathology, the goal of this study was to determine the long-term effects on the cholinergic anti-inflammatory network in both the central nervous system and the periphery in our previously established rat model of GWI, consisting of PB treatment in combination with repeated restraint stress (RRS). Specifically, we used in vivo microdialysis to assess cholinergic neurochemistry in the prefrontal cortex (PFC) and hippocampus following a mild immune challenge (lipopolysaccharide; LPS) and measured inflammatory markers in these brain regions and in plasma 3 months after the treatment paradigm. We found that PB treatment produces a long-lasting attenuation of the cholinergic response to LPS in both the PFC and hippocampus and this is accompanied by neuroinflammation in these regions. Interestingly, this attenuation in cholinergic neurochemistry is not seen when PB treatment is combined with RRS, but this combination also produces neuroinflammation. In the periphery, PB treatment, alone and in combination with RRS, elicited dysregulation of the cholinergic anti-inflammatory network. In this regard, while LPS-induced increases in plasma levels of IL-6 were reduced in PB-treated

rats, these rats exhibited an unexpected enhancement of plasma levels of C-reactive protein. Collectively, these findings provide evidence that neuroinflammation may be a mechanistic mediator of the progression of the neurochemical and neurocognitive deficits in GWI.

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Poster

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Program #/Poster #: P598.03

Topic: F.04. Stress and the Brain

Support: NARSAD Young Investigator Award (GEH)

Title: Of Mice and Wo/Men: Translating the Relationship Between Cytokines, Stress and Depression

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Abstract: The underlying biological mechanisms of depression in the brain differ between men and women. Recent work has demonstrated the peripheral immune system influences the brain's response to stress and subsequent behavior. The peripheral immune system is altered in patients with depression and may contribute to sex differences in the prevalence and symptoms of depression. We took an unbiased approach to examine differences in plasma cytokine expression between pre-menopausal women and age-matched men with depression. We found that women who failed to respond to at least two rounds of treatment with traditional anti-depressants had a unique and stronger immune response than women who had only been treated for an initial bout of depression, or men in either stage. We modeled these changes in immune activity with mouse stress paradigms to uncover the relationship between peripheral cytokine expression and behavior. We used Multiplex ELISA to quantify plasma expression levels of 29 different cytokines in plasma samples from men and women diagnosed with depression (treatment resistant vs. non treatment resistant), as well as from mice of both sexes following chronic variable stress for both 6 and 28 days. Female mice exhibited a stronger immune response to stress than males. Four Core Genotype Mice (FCG) are bred to either express the Sry gene on an

autosome or to have it deleted, which allowed us to investigate the contribution of gonadal sex versus chromosomal sex. Mice with female chromosomes had either male gonads or female gonads, and mice with male chromosomes had either female gonads or male gonads. There is a behavioral sex-difference in wild-type mice following 6 days of variable stress, in which females are stress susceptible, but males remain resilient. The FCG mice were run through the same 6-day variable stress paradigm. In the forced swim test, which measures active versus passive coping, karyotypically female (XX) mice with male gonads (testes) showed resilience equal to that of XY mice with proper testes development. These results confirm sex-specific immune system activation in depression, and demonstrate the translational potential for using stress paradigms in mice to model specific immune responses. Results suggest that the immune response to stress is influenced by both chromosomal and gonadal sex.

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Poster

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Program #/Poster #: P598.04

Topic: F.04. Stress and the Brain

Support: Fondation du CHU de Québec

Title: The contribution of interleukine-1β in susceptibility to psychological trauma in a mouse model of PTSD

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Abstract: Background and hypothesis: Posttraumatic stress disorder (PTSD) is a psychiatric condition that affects over 9% of the general population with rates up to 20% in veterans. PTSD dramatically decreases patients' quality of life and can persist for multiple years. The only FDA-approved drugs available to treat PTSD are antidepressants and have very limited effectiveness. It is therefore urgent to better understand the pathophysiological mechanisms of PTSD in order to develop new therapeutic avenues. Several studies support a role of inflammation in the development of PTSD symptoms, but the mechanisms underlying immune function and PTSD risk remains unclear. In a unique mouse model of PTSD, we found increased plasma and brain levels of the pro-inflammatory cytokine interleukin-1β (IL-1β). However, the contribution of this cytokine in the development of PTSD, as well as its underlying mechanisms, remains to be

investigated. We hypothesized that IL-1β is critical for the onset of PTSD-like phenotype.
Methods: To test this hypothesis, male and female wild-type (WT) and IL-1β-deficient (IL-1βKO) mice were exposed to predator stress (physical contact with a cat for 10 min). Seven to nine days after stress, anxiety-like and PTSD-like behaviors, including avoidance of trauma-related cues were evaluated using the open field, light-dark box, and trauma-reminder tests. Ten days after exposure, brain tissues were collected to measure the levels of the inflammatory markers (C-reactive protein, interleukin-6 and tumor necrosis factor-α) using ELISAs.
Results: In both males and females, predator stress increased anxiety-like and PTSD-like behaviors in WT mice ($p < 0.001$, vs. non-stressed WT mice), but did not induce the same phenotype in IL-1β-deficient mice. We observed no effect of stress or IL-1β deficiency on neuroinflammation.
Conclusion: These results suggest that IL-1β plays a causal role in the development of PTSD, and therefore in vulnerability and resilience to psychological trauma. Data on plasma levels of inflammatory markers shortly (24 hrs) after stress exposure, as well on the integrity of the blood-brain barrier (BBB) will also be presented to provide additional key insights on the mechanisms underlying the association between IL-1β and PTSD risk.

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Poster

P598. Stress and Neuroimmunology

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Program #/Poster #: P598.05

Topic: F.04. Stress and the Brain

Title: A mediating role for microglia in the sex-dependent cognitive and neurostructural consequences of neonatal and juvenile adversity

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Abstract: Early life is rife with sensitive periods during which aberrant experiences can have lasting effects on plasticity and cognitive development. Formation of perineuronal nets (PNNs) - specialized extracellular structures that preferentially enwrap fast-spiking parvalbumin (PV)-expressing interneurons - is essential for proper neurodevelopment. We have previously observed female-specific prefrontal cortex (PFC) PNN degradation following multiple hits of developmental adversity. Importantly, microglia - the main immune cells in the brain - have been

implicated in regulating cell plasticity and PNN modification in healthy and disease states. To determine whether microglial presence in the neonatal brain mediates the sex-dependent behavioral and neurostructural consequences of early life adversity, pups underwent bilateral intracerebroventricular infusions of liposomal clodronate (LC) or vehicle liposomes at postnatal day (P)2 to transiently deplete microglia. Pups were then exposed to maternal separation (MS) 4h per day from P2-20 (or control rearing), followed by juvenile social isolation (SI) from P21-35 (or standard pair-housing). In adulthood (P70-71), working memory (spontaneous alternation in the Y-maze), social preference, and social recognition was assessed, followed by quantification of the density and structural integrity of PFC PV+ PNNs, as well as the transcriptional factor ΔFosB, that regulates neuronal and behavioral plasticity. We found female-specific deficits in social and working memory following MS that was prevented by LC treatment. Notably, juvenile SI improved working memory, but exerted the opposite effect in LC-treated females. Here, we reveal sex-specific neurostructural and cognitive consequences of early life adversity at distinct developmental stages that are mediated by developmental neuroimmune activity.

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Poster

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Program #/Poster #: P598.06

Topic: F.04. Stress and the Brain

Support: NIH - 1R01MH123545-01
NIH - 1F32MH123051-01

Title: Microglial P2Y12 mediates stress-induced structural remodeling of neurons in the prefrontal cortex and associated behavioral deficits

Authors: *J. L. BOLLINGER, D. T. DADOSKY, I. L. RAINER, S. C. WOODBURN, J. K. FLURER, E. S. WOHLEB;
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Abstract: Recent studies demonstrate that chronic unpredictable stress (CUS) drives microglia-mediated neuronal remodeling, which contributes to synapse loss in the prefrontal cortex (PFC) and cognitive-behavioral dysfunction. Despite this work, it remains unclear what mechanisms guide microglia-neuron interactions in stress. Recent studies show that neuronal activity-dependent purinergic signaling directs microglia-synapse interaction via the purinergic receptor,

P2Y12, which is selectively expressed by microglia in the brain. Thus, microglial P2Y12 may contribute to stress-induced structural remodeling of neurons in the PFC and associated behavioral consequences. To investigate this, we used genetic (*P2ry12-/-*) or pharmacological (clopidogrel, ticagrelor) approaches to block P2Y12 signaling in the context of CUS. Various behavioral, physiological, and cytometric endpoints were analyzed. Both P2Y12-deletion and treatment with clopidogrel prevented stress-induced increases in forced swim test immobility and attenuated deficits in temporal object recognition. Genetic ablation of P2Y12 or treatment with clopidogrel diminished microglial P2Y12 and altered surface receptor levels of CX3CR1, CSF1R, and CD11b, independent of CUS. Blocking microglial P2Y12 prevented stress-induced increases in microglial CD68 and the proportion of microglia with neuronal inclusions, and attenuated dendritic spine loss in the PFC. Together, these findings indicate that microglial P2Y12 contributes to stress-induced dendritic remodeling in the PFC and subsequent behavioral deficits.

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Poster

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Topic: F.04. Stress and the Brain

Support: R01 MH118237)
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Title: Age-dependent effects of acute lipopolysaccharide administration

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Abstract: The phenomena of sickness behavior is not well understood, but is a behavioral response driven by the immune system in response to a pathogen that is usually transient in nature. Lipopolysaccharide (LPS) administration can induce sickness behaviors at specific doses through stimulation of the immune system and the HPA axis. Though the immune system

matures, LPS effects have not been well elucidated during adolescence. Here, we explored the dose-dependent effects of acute LPS during early adolescence and adulthood. Rat subjects were injected with a single dose of LPS (0.1, 1.0, 10.0, 100.0 µg/kg) or vehicle (0.9% saline) and placed in an open field to assess general locomotion. Serum and brains were harvested following behavior to assess peripheral inflammation and neuronal activation. In adults, LPS had a main effect on distance traveled and increased immobility when administered at 100.0 µg/kg. In adolescents, 100.0 µg/kg of LPS decreased distance traveled but immobility time did not change across LPS doses. Though cytokine production did not differ between age-groups administered with 0.1 and 1.0 µg/kg LPS, the low doses produced age-dependent effects on open field distance traveled. Adult 0.1 and 1.0 µg/kg LPS treated subjects exhibited hyperactivity and adolescent behavior did not change. 100.0 µg/kg LPS increased immobility in both age groups, but trended towards a larger effect in adults relative to adolescents. Collectively, these results suggest that at similar peripheral inflammatory states, LPS-induced inflammation has an age-dependent effect on sickness-like behavior.

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Poster

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Topic: F.04. Stress and the Brain

Support: NIH R01-MH119670
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Title: Brain endothelia activated by social stress increase prostaglandin E2 signaling, neuronal activation, and augment anxiety-like behavior

Authors: *W. YIN, R. G. BILTZ, E. J. GOODMAN, S. P. SWANSON, J. F. SHERIDAN, J. P. GODBOUT;
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Abstract: Chronic psychosocial stress can increase neuropsychiatric complications including anxiety and depression. We have reported that Repeated Social Defeat (RSD) in mice increased the accumulation of inflammatory monocytes within the brain vasculature. This accumulation of cells with RSD corresponded with increased interleukin (IL)-1R mediated activation of endothelial cells and the augmentation of anxiety-like behavior after RSD. One unknown, however, is the role of IL-1 activated endothelia in regulating the behavioral and physiological

responses to RSD. Thus, the goal of this study was to determine the RNA profile of activated endothelia and to begin to delineate the pathway by which with endothelial cells communicate with neurons to influence behavioral responses. In the first experiments, mice were exposed to RSD and brain endothelial mRNA profiles were determined using RiboTag immunoprecipitation and RNA sequencing. RSD increased the active translation of cell adhesion molecules (*Icam1*, *Vcam1*), innate inflammatory proteins (*Lrg1*, *Lcn2*, *Ackr1*), and cyclooxygenase-2 (*Ptgs2*/COX-2). In the prelimbic cortex, activated endothelia reduced expression of tight junction proteins *Cldn5* and *Mfsd2a*. Overall, these RNA-sequencing data showed an increased adhesion molecule and prostaglandin signaling signature of brain endothelia activated by RSD. Based on these data, we next examined the role of prostaglandin (PG)E2 in the peripheral and central immune responses to stress. In these experiments, mice received celecoxib, a selective COX-2 inhibitor, prior to each cycle of RSD. As expected, RSD increased the PGE2 concentration in the brain and this increase was abrogated by inhibition of COX-2. Moreover, inhibition of COX-2 blocked anxiety-like behavior and working memory deficits after RSD. Inhibition of COX-2 also reduced the stress-induced activation of neurons in the paraventricular nucleus of the hypothalamus and increase in IL-6 in the plasma. Taken together, synthesis of PGE2 after RSD, in part by endothelial cells, influences neuronal activity and behavior.

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Poster

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Topic: F.04. Stress and the Brain

Support: NIH R01 MH62044
GSU Brains and Behavior Seed Grant

Title: Consumption of dietary emulsifiers increases social stress sensitivity in mice

Authors: *A. R. ARNOLD[1], K. LAKHANI[1], T. WILKINSON[3], K. L. HUHMAN[2];
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Abstract: Chronic low-grade inflammation and stress exposure are key contributing factors in the etiology and progression of many neuropsychiatric disorders. Dietary emulsifiers are commonly added to processed foods and are classified by the Food and Drug Administration as safe. Recently, however, it has been revealed that these additives can cause inflammation in the gut and may even increase baseline anxiety-like behavior. It is not known, however, whether emulsifier-treated mice exhibit normal behavioral and hormonal responses to stress. In this study,

we tested the hypothesis that chronic consumption of either polysorbate 80 (P80) or carboxymethylcellulose (CMC), two common emulsifiers, sensitizes the behavioral response to social stressors. C57Bl6/J mice (n=10 per group) were subjected to social defeat (3, 5 min pairings with aggressive CD1 opponent with a 5 min break between pairings) or no defeat after 12 weeks of emulsifier (1% in drinking water; P80 or CMC) or plain water consumption. 24 hours post defeat social avoidance testing was conducted. Our results show that when subjected to social defeat, emulsifier-treated mice show increased social avoidance and greater increases in the stress hormone corticosterone compared to water-treated controls. Subsequently, we investigated the role of the COX pathway as a possible inflammatory mechanism driving emulsifier induced social stress sensitivity. A previous RNA-Seq study conducted in our lab showed increased differential expression of PTGS2 (COX-2), a gene that codes a protein responsible for the conversion of arachidonic acid to proinflammatory prostaglandins, in the amygdala following emulsifier consumption. We therefore tested the hypothesis that increased inflammation, specifically through the COX pathway, is a mechanism driving emulsifier-induced stress sensitivity. Groups were as described above, but mice were also divided into aspirin (25mg/kg/day) intervention groups. Aspirin was added to drinking water or respective emulsifier at week six for a duration of six weeks (n=7 per group). Results suggest that aspirin, a COX pathway inhibitor, may block the increased social avoidance observed in emulsifier-treated mice. Taken together, these data demonstrate that ingestion of dietary emulsifiers at concentrations commonly ingested by humans increases sensitivity to social stress and that the COX pathway may be a prime mechanistic candidate by which emulsifiers increase sensitivity to social stress. [Supported by NIH R01 MH62044 and a GSU Brains and Behavior Seed Grant to KLH. Views expressed are those of the authors and do not reflect a position of the NIH or GSU.]

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Poster

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Topic: F.04. Stress and the Brain

Support: VA Merit Award
NIH DaCCoTA
SD BRIN
SPURA

Title: Molecular Mechanisms of Reduced Ethanol Drinking in Calcineurin-Mediated Immunosuppression Treated Rodents

Authors: *P. J. RONAN[1,2], S. A. FLYNN[2], T. P. BERESFORD[3];
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Abstract: We have shown that calcineurin (CLN)-meditated immunosuppressants reduce alcohol intake in mice. Calcineurin plays key roles in reward, stress, and neuroinflammatory signaling. Ethanol use causes enhanced neuroinflammation and increased stress signaling. Similarly, stress also induces neuroinflammatory responses. Here we sought to determine the effects of cyclosporine A (CsA) on stress-induced neuroinflammatory markers in brain. Rats (n=8) were given either CsA (30 mg/kg, I.P.) or vehicle, exposed to 30 minutes of restraint stress and returned to their home cage for 90 minutes. Brains were rapidly dissected and frozen in isopentane on dry ice. The CeA and PVN were microdissected from 300 um frozen sections and qRT-PCR was performed. Overall, Cyclosporine inhibited the expression of a wide range of neuroinflammatory markers in these regions including cytokines such as IL-2, IL-1β, IL-6, TNFα; markers of glial activation: CD45 and Iba-1; chemokine and chemoattractant molecules such as CCR2 and CCL2; as well as other inflammatory signaling molecules such as COX-2. Some of the largest effects were seen on IL-1 β and IL-6 expression in both CeA and PVN. While CsA inhibited the expression of CD45 and iba1 in the CeA, in the PVN these effects were striking. This suggests that stress rapidly induces glial activation in these regions which is inhibited by CsA. Together these data suggest that rapid, stress-induced neuroinflammatory signaling is attenuated by inhibition of CLN activity. This has implications for the treatment of multiple disorders in which stress is an etiological or maintenance factor such as PTSD and AUD.

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Poster

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Topic: F.04. Stress and the Brain

Support: NIH Grant R01DK047320
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UH Manoa - Undergraduate Research Opportunities Program
Douglas S. Yamamura Scholarship

Title: Investigating the interactions between selenium and glucocorticoids in a murine hypothalamic cell line

Authors: *D. J. TORRES[1], K. J. AN[1], A. N. HANATO[1], M. W. PITTS[2], K. W. HUI[1,3], S. A. REMIGIO[1,4], J. KIM[1], L. A. SEALE[1], M. J. BERRY[1];
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Abstract: Glucocorticoids are a class of steroid hormones produced by the adrenal gland in response to stress. Chronic elevation of glucocorticoids is known to cause neurological and metabolic impairments. Glucocorticoids are commonly prescribed as anti-inflammatories and the side effects of long term administration include overeating and excess weight gain. Recent reports suggest that selenium, an essential micronutrient with antioxidant capabilities, may help combat the negative impact of glucocorticoids on the brain. In this study, a mouse hypothalamic cell line was treated with corticosterone (CORT), the primary active glucocorticoid in mice, to investigate the neurological underpinnings of glucocorticoid-induced metabolic dysfunction. Additionally, the potential protective role of selenium was assessed. Seven days of CORT potentiated tunicamycin-induced endoplasmic reticulum stress (ER stress), which was correlated with a non-transcriptional downregulation of the selenium-containing selenoprotein M (SELENOM). Supplementation with excess selenium prevented SELENOM downregulation and reversed the exacerbating effect of CORT on ER stress. These data suggest that chronic CORT may disrupt hypothalamic function by decreasing SELENOM expression, thereby making neurons more susceptible to ER stress. Moreover, selenium supplementation may help mitigate this effect.

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Poster

P598. Stress and Neuroimmunology

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P598.12

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

Support: CDMRP W81XWH-19-1-0725

Title: Assessing neuroinflammation in Veterans with Gulf War Illness: A pilot study using whole-brain magnetic resonance spectroscopy

Authors: *C. JONES[1], A. MAUDSLEY[2], S. SHERIFF[2], E. WETZEL[1], J. ROBINSON[1], J. YOUNGER[1];
[1]Univ. of Alabama, Birmingham, Birmingham, AL; [2]Radiology, Univ. of Miami, Miami, FL

Abstract: Background: Nearly one-third of the Veterans who served in the 1991 Persian Gulf War suffer from a chronic condition involving fatigue, pain, cognitive impairment, respiratory issues, gastrointestinal complaints, and/or mood disorders. Mounting evidence suggests that this condition, named Gulf War Illness (GWI), results from abnormal neuroinflammatory processes. This pilot study is the first to use a whole-brain magnetic resonance spectroscopic imaging (MRSI) technique to assess markers of widespread neuroinflammation in GWI.
Methods: Whole-brain MRSI was used to quantify four neuroinflammatory markers across the brains of Veterans with GWI, and healthy Gulf War Veterans (HGWV). The four measured metabolites of myo-inositol (MI), lactate, choline, and N-acetyl-aspartate (NAA) were expressed as ratios over Creatine (Cr). For this pilot investigation, data from 5 male HGWV and 5 male GWI, ages 49-62, were analyzed with independent sample t-tests (significance threshold of $p < 0.05$). Higher levels of MI, choline, and lactate, and lower levels of NAA, were expected in the GWI group, indicating inflammation.
Results: Our preliminary analyses revealed 15 significant metabolite group differences in 14 out of 47 brain regions. The GWI group showed elevated lactate/CR in the left Rolandic operculum, the right insula, the right calcarine sulcus, and the right caudate nucleus. The GWI group also showed lower Choline/Cr in the left frontal cortex, the left Rolandic operculum, the right mid cingulum, bilateral posterior cingulum, the left postcentral gyrus, the left parietal cortex, bilateral precuneus, and the left paracentral lobule. There was one additional significant finding of lower NAA in the left anterior cingulum in the GWI group.
Interpretation: These preliminary findings provide additional support for the hypothesis that GWI involves neuroinflammation. Low choline in the brain may also indicate abnormalities in acetylcholine synthesis. If replicable in larger samples of Gulf War Veterans, whole-brain MRSI may be used to identify targets for improved treatments.

Disclosures: C. Jones: None. **A. Maudsley:** None. **S. Sheriff:** None. **E. Wetzel:** None. **J. Robinson:** None. **J. Younger:** None.

Poster

P607. Neural Mechanisms Underlying Stress-Reactivity

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P607.01

Topic: F.04. Stress and the Brain

Support: AHA Predoctoral Fellowship to D. Schaeuble
NIH grants HL122454 and HL150559 to B. Myers

Title: Sex differences in cortical-hypothalamic circuitry mediating stress reactivity

Authors: *D. SCHAEUBLE, T. WALLACE, B. MYERS;
Biomed. Sci., Colorado State Univ., Fort Collins, CO

Abstract: Endocrine and autonomic stress responses adapt the body to real or perceived threats by engaging physiological pathways to maintain homeostasis. Unfortunately, the burden of prolonged stress contributes to maladaptive stress responding, increasing risk for pathologies including cardiometabolic disorders. However, the circuit mechanisms integrating endocrine and autonomic stress responses have yet to be defined. Our previous studies found that glutamate neurons of the infralimbic cortex (IL) have sex-specific effects on endocrine and cardiovascular stress reactivity. While the IL is important for coordinating behavioral and physiological responses to stress, these cortical cells do not directly innervate the secretory or pre-autonomic cells that initiate endocrine and autonomic stress responses. However, we previously found that the posterior hypothalamus (PH) receives dense stress-activated inputs from the IL. Thus, the current study tests the hypothesis that IL glutamate release in the PH mediates sex-specific effects on stress integration. To test this hypothesis, adult male and female Sprague-Dawley rats received stereotaxic injections in the IL of a viral-packaged construct expressing channelrhodopsin-2 and bilateral fiber optics targeting the PH to stimulate IL synapses in the PH. After recovery and acclimatization to handling, rats were tested in the real-time place preference paradigm to examine behavioral preference or aversion for circuit stimulation. These data indicate that IL-PH signaling is preferred by males and that females have neither preference nor aversion for IL-PH stimulation. Additionally, acute restraint was used to examine glucose and corticosterone responses to stress. Results found that IL synapses in the PH decrease stress-induced glucose mobilization without affecting corticosterone in male rats. In contrast, IL-PH stimulation in females increases corticosterone responses to restraint but does not affect glucose mobilization. Furthermore, non-invasive assessment of hemodynamics indicate that IL-PH stimulation increases heart rate in females but not males. Thus, the IL-PH circuit increases place preference and reduces glucose responses to stress in males, while increasing corticosterone stress responses and heart rate in females. Taken together, these data suggest that the PH represents a key node for sex-specific integration of descending cortical signals with endocrine and autonomic stress reactivity. Ultimately, sex-specific delineation of stress-regulatory neurocircuitry may provide insight into the differential impact of stress pathologies in men and women.

Disclosures: D. Schaeuble: None. **T. Wallace:** None. **B. Myers:** None.

Poster

P607. Neural Mechanisms Underlying Stress-Reactivity

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P607.02

Topic: F.04. Stress and the Brain

Support: NHLBI Diversity Supplement
NIH R01 HL150559

Title: Prefrontal-medullary circuit activation attenuates stress reactivity

Authors: *S. A. PACE, E. LUKINIC, T. WALLACE, D. SCHAEUBLE, J. MOORE, B. MYERS;
Biomed. Sci., Colorado State Univ., Fort Collins, CO

Abstract: Organismal survival and adaptation to stress rely on brainstem catecholaminergic neurons. In particular, catecholaminergic neurons in the rostral ventrolateral medulla (RVLM) drive sympathetic activity and enable physiological adaptations, including vasoconstriction, corticosterone release, and glycemic mobilization. However, it is unclear how brain regions involved in the cognitive appraisal of stress regulate the activity of RVLM neurons. Our previous studies found that the rodent infralimbic prefrontal cortex (IL) integrates behavioral and physiological responses to stress. Thus, a potential IL-to-RVLM connection would represent a crucial link between stress appraisal and sympathetic reactivity. In the current study, we investigated a direct IL-to-RVLM circuit by targeting a genetically-encoded anterograde tracer under the control of the CaMKIIα promoter to the IL of adult male and female rats. Analysis revealed that IL terminals apposed RVLM neurons expressing the catecholamine-synthesizing enzyme, dopamine beta-hydroxylase. Further, IL input to catecholamine cells was widespread throughout the rostral-to-caudal extent of the VLM in both male and female rodents. Quantification of innervation density revealed that males had a larger proportion of VLM catecholamine neurons receiving IL inputs than females. Additionally, IL appositions were identified on GABAergic and glycinergic neurons in both sexes. Accordingly, we hypothesized that IL projections may activate local RVLM inhibitory cells to limit sympathetic output. To test this hypothesis, we injected a viral vector coding for channelrhodopsin-2 (ChR2) in the IL of males and females. Next, a fiber optic cannula was implanted dorsal to the RVLM to evoke IL synaptic glutamate release. Animals then received photostimulation during restraint stress with blood sampled to determine stress reactivity. Compared to controls, male rats expressing ChR2 on IL terminals had suppressed glycemic stress responses. In contrast, stimulation of the IL-RVLM circuit in females did not affect glucose mobilization. However, ChR2 decreased corticosterone responses to stress relative to control rats in both sexes. Thus, both male and female rats have a direct circuit from the IL portion of the mPFC to catecholamine-synthesizing cells of the RVLM that limits glucocorticoid stress responses, likely through the activation of local inhibitory neurons. However, the density of this circuit is greater in males, potentially accounting for reduced glycemic responses. Ultimately, excitatory/inhibitory balance at IL synapses in the RVLM may be critical for the health consequences of stress.

Disclosures: S.A. Pace: None. **E. Lukinic:** None. **T. Wallace:** None. **D. Schaeuble:** None. **J. Moore:** None. **B. Myers:** None.

Poster

P607. Neural Mechanisms Underlying Stress-Reactivity

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P607.03

Topic: F.04. Stress and the Brain

Support: HL122454
HL150559

Title: Chronic stress impacts on ventromedial prefrontal encoding of social behavior, stress responding, and reward are stimulus and sex dependent.

Authors: *T. WALLACE, B. MYERS;
Biomed. Sci., Colorado State Univ., Fort Collins, CO

Abstract: The rodent infralimbic cortex (IL) is anatomically homologous to Brodmann area 25, a prefrontal region implicated in depressive etiology and treatment. Thus, understanding how specific neural populations within the IL encode and modulate depression-relevant behaviors is critical for developing more specific neuro-behavioral interventions. In the current study, we hypothesized that chronic stress reduces the in vivo activity of excitatory IL pyramidal neurons based on prior ex vivo and histological studies suggesting enhanced IL inhibition. To address this hypothesis, we utilized a fluorescent calcium indicator to record the activity of IL glutamatergic pyramidal neurons during behavioral tests. In brief, adult male and female rats received unilateral injections in the IL of an adeno-associated virus carrying a construct expressing the fluorescent calcium indicator GCaMP6s under the pyramidal neuron promoter CaMKIIα. In the same surgery, a fiber optic cannula was implanted in the IL for the collection of calcium-induced changes in GCaMP fluorescence. Rats were then assigned to either a chronic variable stress (CVS) group or a stress-naïve group. Following two weeks of CVS or handling, rats were exposed to a modified social assay, 3-day repeated restraint stress, and hedonic food reward. Our results indicate that both male and female IL glutamatergic neurons increase activity during both novel object and social interactions. However, CVS increased activity during objection interactions in females while decreasing activity during social interactions in males. During repeated restraint, both male and female IL glutamatergic neurons had increased activity. Further, CVS sensitized male and female corticosterone responses to restraint while increasing glucose responses in males only. In addition, CVS-exposed males had diminished IL activity during the last 5 minutes of restraint compared to stress-naïve rats on the first day, though this effect was gone by the third day. Both males and females had increased IL activity associated with food reward (chocolate) acquisition. In males but not females, CVS exposure increased the IL food-acquisition response, an effect partially accounted for by reduced food intake. In summary, chronic stress appears to alter IL activity in behaving males more than females. Ongoing studies aim to determine if ovarian hormones protect females from CVS-induced activity changes. Altogether, our data indicate that IL glutamatergic neurons are responsive to novel non-social stimuli, novel social stimuli, acute stressors, and food reward acquisition. Further, CVS alters this activity differently based on stimulus and sex.

Disclosures: T. Wallace: None. **B. Myers:** None.

Poster

P607. Neural Mechanisms Underlying Stress-Reactivity

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P607.04

Topic: F.04. Stress and the Brain

Support: R01 MH122749 02
R00 MH109627 04

Title: Crh release from sparse “eccentric” medium spiny neurons in the nucleus accumbens is required for adaptive novelty responses

Authors: *C. L. PETERSEN[1], J. J. STOLLEY[1], M. A. FUSARO[1], A. E. INGEBRETSON[1], E. A. SOUTER[1], M. BOCARSLY[2], D. DA SILVA E SILVA[3], V. A. ALVAREZ[4], J. C. LEMOS[1]; [1]Neurosci., Univ. of Minnesota, Minneapolis, MN; [2]Natl. Inst. on Alcohol Abuse and Alcoholism, NIH, Bethesda, MD; [3]NIH, Rockville, MD; [4]Lab. on Neurobio. of Compulsive Behaviors, Natl. Inst. on Alcohol Abuse and Alcoholism, Bethesda, MD

Abstract: Corticotropin releasing hormone (CRH) is expressed broadly across the brain and is released in response to stressor exposure as well as other salient environmental stimuli. Within the nucleus accumbens (NAc), a crucial hub for limbic and cortical convergence and a key regulator of motivated behaviors, resides a population of CRH neurons whose neurochemical identity and function remains unknown. Here, we characterize the neurochemical identity and the electrophysiological properties of CRH neurons in the NAc. First, we used fluorescent *in situ* hybridization (FISH) to visualize markers for direct or indirect pathway medium spiny projection neurons (dMSNs vs. iMSNs, respectively) as well as different accumbal interneurons. We found that >85% of *Crh* neurons coexpressed *Drd1* in male mice where only 46% of *Crh* neurons coexpressed *Drd1* in females. Interestingly, only 25% of male and 46% of female *Crh* positive neurons coexpressed the iMSN marker *Drd2*. Together CRH neurons in the male NAc are predominantly dMSNs, whereas in females they are predominantly iMSNs. Consistent with this neurochemical characterization, electrophysiological recordings from CRH-Reporter mice indicate that putative NAc CRH+ neurons have both classic MSN and atypical electrophysiological signatures which are consistent with a recently described “eccentric” MSN population. Next, we deleted *Crh* from the NAc using viral transduction of CRE:eGFP into $Crh^{loxP/loxP}$ male and female mice. Conditional knockout (cKO) mice decreased exploration during the first minute of open field trials, which along with a modest disruption in locomotion as the session continued suggest reduced habituation to the arena. Further, cKO mice increased entries into a zone containing a novel object, but not into a zone containing palatable food. We also found an interaction where cKO females increased sucrose preference relative to controls, whereas male cKO mice tended to decrease sucrose preference. Characterizing CRH neurons in the NAc sheds insight into the contribution of this discrete population to naturalistic behaviors,

with the goal to further understand how CRH signaling may be effected during stress-related psychopathologies.

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Poster

P607. Neural Mechanisms Underlying Stress-Reactivity

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P607.05

Topic: F.04. Stress and the Brain

Support: MH109627-04

Title: Investigating CRF receptor type 1 and estrogen receptor alpha regulation of cholinergic interneurons in the nucleus accumbens shell

Authors: *A. E. INGEBRETSON, K. L. OLSON, P. G. MERMELSTEIN, J. C. LEMOS; Univ. of Minnesota, Minneapolis, MN

Abstract: Sex differences in stress encoding as well as the distribution and function of the stress-associated peptide corticotropin-releasing factor (CRF) throughout the brain have been well documented. However, the mechanisms through which ovarian hormones and CRF signaling converge on cells and circuits of the brain modified by stress is unclear. The nucleus accumbens (NAc) in the ventral striatum is a key region that integrates limbic and cortical inputs to shape reward-related behavior. CRF receptor type 1 (CRFR1) is present within the NAc on cholinergic interneurons (ChIs), large aspiny interneurons that constitute 1% of cells in the NAc but broadly modulate striatal circuitry. Activation of CRFR1 robustly potentiates the firing rate of ChIs, indicating that CRF can regulate striatal circuitry through modulating ChIs. Here, we investigated sex- and estrous cycle-dependent differences in CRF regulation of NAc ChIs. Using the fluorescent *in situ* hybridization technique RNAscope, we found that 95% of ChIs in female mice expressed *Crhr1* compared to 81% in male mice. Additionally, we probed for the estrogen receptor *Esr1* (ERα) since rapid membrane-initiated estrogen receptor signaling can participate in modifying neuronal activity and plasticity in the NAc. In female mice, 24% of ChIs expressed both *Crhr1* and *Esr1*, while only 15% co-expressed both markers in male mice. Across stages of the estrous cycle, we observed significant differences in the expression of *Crhr1* and *Esr1*. We next performed cell-attached patch-clamp electrophysiology recordings in ChIs to investigate sex and estrous cycle differences in the CRF-induced enhancement of ChI firing. We observed that ChIs from females displayed lower firing rates at baseline compared to males (females: 2.52 Hz; males: 3.62 Hz), but there were no differences between sexes in the sensitivity of ChIs to CRF.

Interestingly, ChIs from estrus stage females displayed lower CRF-induced firing rates compared to cells from other stages, suggesting that low levels of circulating estrogen during estrus may dampen ChI responsivity to CRF. Current investigations are focused on examining the temporal firing pattern of ChIs, which are characterized by tonic or bursting patterns. Our findings indicate that estrus stage females at baseline display an increased prevalence of ChIs with bursting patterned activity. Understanding the action of estrogen and its receptors on cells and circuits of the brain modified by stress may provide improved treatment options for neuropsychiatric disorders that differ in prevalence or severity by sex.

Disclosures: A.E. Ingebretson: None. **K.L. Olson:** None. **P.G. Mermelstein:** None. **J.C. Lemos:** None.

Poster

P607. Neural Mechanisms Underlying Stress-Reactivity

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Program #/Poster #: P607.06

Topic: F.04. Stress and the Brain

Support: NIH R01 MH073136
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NIH T32 MH119049-02
UCI Undergraduate Research Opportunities Program

Title: High levels of hippocampal estrogen contribute to the memory-impairing effects of multiple concurrent acute stresses in female and male mice

Authors: *R. E. HOKENSON, A. K. SHORT, Y. H. ALAM, S. JUNG, Y. CHEN, A. PHAM, S. A. SAMRARI, C. JANG, C. M. GALL, T. Z. BARAM;
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Abstract: Background: Chronic stress may provoke memory problems, and these vary by sex. More recently memory problems are being recognized following short, traumatic/ stressful events, including mass shootings, assault, or natural disasters, which comprise physical, social, and emotional components. We have established in male mice that multiple acute concurrent stresses (MAS) impair hippocampus dependent spatial memory in the object location and spatial Y-maze, and destroy dorsal CA1 synaptic integrity. In females, MAS impair memory in an estrous cycle-dependent manner, specifically, impairing hippocampal memory in early-proestrous mice, characterized by high levels of estrogen, yet sparing memory of mice stressed during estrus (low estrogen). Notably, hippocampal estrogen levels are higher in males and proestrous females, the mice that are vulnerable to MAS compared to resilient estrous females. Surprisingly, these findings suggest that high levels of hippocampal estrogen may augment

MAS-induced memory disruptions.
Methods: To understand how differences in the relative levels of systemic and hippocampal estrogen may promote susceptibility or resilience of memory to MAS, we examined the impacts of MAS when estrogen activity was pharmacologically blocked, either by blocking estrogen production or selectively blocking estrogen receptor (ER) subtypes. Pharmacological agents were delivered systemically or directly in hippocampus through indwelling cannulae. We quantified estrogen levels by enzyme-linked immunosorbent assay (ELISA) and mass spectrometry.
Results: Blocking estrogen production by administering an aromatase inhibitor for one week leading up to stress prevented memory deficits after MAS compared to vehicle-treated males or proestrous females. In a separate experiment, blocking estrogen receptor (ER) activation by administering ERα or ERβ antagonists, MPP and PHTPP respectively, 90 minutes prior to stress, protected mice from memory impairments. These findings implicate a role of estrogen in promoting memory disruptions following MAS. Studies are underway to expand these findings to hippocampus-specific manipulations.

Disclosures: R.E. Hokenson: None. **A.K. Short:** None. **Y.H. Alam:** None. **S. Jung:** None. **Y. Chen:** None. **A. Pham:** None. **S.A. Samrari:** None. **C. Jang:** None. **C.M. Gall:** None. **T.Z. Baram:** None.

Poster

P607. Neural Mechanisms Underlying Stress-Reactivity

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P607.07

Topic: F.04. Stress and the Brain

Title: Acute prolactin increases stress-induced passive coping and corticosterone responses but reduces anxiety-like behavior in ovariectomized female rats

Authors: *J. MEDINA, J. L. WORKMAN;
Psychology, Univ. at Albany State Univ. of New York, Albany, NY

Abstract: Beyond its role in promoting lactation, prolactin (PRL) influences multiple aspects of physiology and behavior including neural plasticity, stress responses, and anxiety-like behavior in rodent models. Stress is a significant risk factor for depression and abnormalities in the hypothalamic-pituitary-adrenal axis have been implicated in the etiology of depression. Previous work from our laboratory suggests chronic PRL treatment reduces several depression-relevant characteristics including anhedonia, passive coping, and basal corticosterone (CORT) concentrations. We sought to determine whether acute PRL treatment reduces stress-induced depression-like behavior and endocrine responses in female rats. Passive coping was assessed with the forced swim test (FST) across two consecutive days: FST 1 for 15 minutes and FST 2

for 5 minutes. Thirty minutes prior to FST 2, rats received a single injection of PRL (1 mg/kg i.p. or s.c.) or saline vehicle. Blood samples from the tail vein were taken 30 minutes after FST 2 to assess stress-induced CORT concentrations. Acute PRL treatment (i.p.) increased passive coping and post-stress CORT concentrations compared to saline or PRL treatment (s.c.). Locomotor and anxiety-like behaviors were assessed in the elevated plus maze. Acute PRL treatment (i.p.) decreased the latency to first open arm entry compared to saline but had no significant effects on measures of locomotor activity. Additionally, we assessed whether PRL alters acute stress-induced cell proliferation and dendritic spine density. Sections containing the hippocampus were processed for Ki-67, a marker for cell proliferation. Golgi-Cox impregnated tissue sections containing the hippocampus and medial prefrontal cortex (mPFC) were selected for dendritic spine tracing of granule cells (hippocampus) and pyramidal cells (mPFC). Acute PRL did not significantly alter dendritic spine density in the hippocampus or mPFC but tended to increase cell proliferation in the ventral hippocampus. Taken together, these findings further our understanding of neuroendocrine mechanisms underlying stress resilience and susceptibility in females.

Disclosures: J. Medina: None. **J.L. Workman:** None.

Poster

P607. Neural Mechanisms Underlying Stress-Reactivity

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Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P607.08

Topic: F.04. Stress and the Brain

Support: NIDA, Grant 1R15 DA044500-01A1

Title: A crosstalk between Transient Receptor Potential Vanilloid 1 and Cannabinoid Receptor 1 within limbic brain regions regulates depression-like behaviors triggered by stress in rats.

Authors: *W. NORZE, A. RAMOS ROLON, P. MUÑOZ RODRIGUEZ, L. MENDEZ SANTACRUZ, A. RODRIGUEZ LEON, V. SCHLEIER ALBINO, A. ACEVEDO JETTER, M. APONTE REYES, K. TORRES MONTERO, C. MALDONADO VLAAR;
Univ. of Puerto Rico, San Juan, Puerto Rico

Abstract: Clinical studies provide strong evidence that stress is an environmental risk factor that can trigger the onset of several neuropsychiatric disorders such as anxiety and depression in humans. Pre-clinical evidence suggests that the endocannabinoid and endovanilloid system within the brain are important neuronal substrates involved in emotional responses to stress. Specifically, studies have proposed that the Transient Receptor Potential Vanilloid 1 (TRPV1) a member of the Transient Receptor Potential (TRP) superfamily, within the brain regulate anxiety and depression behaviors through its interactions with the cannabinoid receptor 1 (CB1R).

However, little is known about the cellular mechanisms that regulate these receptors' interactions across the brain and its impact in neuropsychiatric disorders. We investigated the role of TRVP1 and CB1R within several brain regions including the medial prefrontal cortex as part of the dopamine mesocorticolimbic system, the hippocampus and the amygdala in anxiety and depression like-behaviors using rats as animal model. To examine the role of these two receptors in depression like-behaviors, male and female Sprague Dawley rats were treated for 4 days with vehicle or FAAH inhibitor URB597 [0; 0.1 ;0.2mg/kg/ip] 2 hours before testing in the forced swim test (FST) for 6 min. Another group were treated for 4 days with either vehicle or Olvanil, a TRPV1 receptor inhibitor [0; 0.3; 0.5 mg/kg/ip] 30 min before testing in the FST for 6 min, a third group of animals were treated for 4 days with either vehicle or URB597 [0; 0.1 ;0.2mg/kg/ip] and Olvanil [0; 0.3; 0.5 mg/kg/ip] 2 hours before testing in the FST for 6 min. Floating and swimming behaviors were observed during the FST session. Our results suggest that URB597 significantly decreased depression like-behavior. In contrast, treatment with olvanil increased depression-like behaviors in the experimental animals when compared to controls. Dual treatment of experimental compounds decreased depression like-behaviors. Ongoing biochemical analysis of the changes in expression of TRPV1 and CB1 receptors following treatment with URB597 and olvanil will support mechanistic explanations for the present behavioral results.

Disclosures: W. Norze: None. **A. Ramos Rolon:** None. **P. Muñoz Rodriguez:** None. **L. Mendez Santacruz:** None. **A. Rodriguez Leon:** None. **V. Schleier Albino:** None. **A. Acevedo Jetter:** None. **M. Aponte Reyes:** None. **K. Torres Montero:** None. **C. Maldonado Vlaar:** None.

Poster

P660. Threat/Fear: Neural Circuits V

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P660.01

Topic: G.01. Fear and Aversive Learning and Memory

Support: NARSAD Young Investigator Grant from the Brain and Behavior Research Foundation (BBRF) 25196

Title: The BNST mediates the expression of two-way signaled avoidance in male rats

Authors: *D. P. GUERRA, J. M. MOSCARELLO;
Texas A&M Univ., College Station, TX

Abstract: Although avoidant behavior is a unifying symptom of many anxiety disorders, its underlying neural circuitry has yet to be elucidated. Here, we report a series of experiments using a signaled active avoidance (SAA) procedure to model avoidant behavior in rats. In SAA, the

subject first acquires an association between a tone (CS) and a shock (US) and then learns to perform an avoidance response during the CS in order to prevent US delivery. Over the course of SAA training, the US becomes less frequent and thus goes from being a certain/imminent threat to a possible/distal threat as the avoidance response is acquired. Though the bed nucleus of the stria terminalis (BNST) has been shown to mediate defensive responses to possible/distal threats, its role in SAA has yet to be examined. We used a chemogenetic approach to test the hypothesis that BNST is necessary for the expression of the avoidance response. Male rats received intra-BNST infusions of AAV containing the gene construct for either the inhibitory hM4Di DREADD or GFP. After recovery, animals received four days of SAA training in order to reach stable levels of avoidance. This was followed by two additional days of SAA training preceded by counterbalanced IP injections of either the DREADD ligand CNO or vehicle. We found that CNO decreased avoidance responses in hM4Di subjects but not in GFP controls, demonstrating that the BNST is necessary for the avoidance response. Because some subjects showed hM4Di expression in the medial septum (MS), we followed up with an anatomical control experiment specifically targeting this region. We found no evidence of a role for MS, indicating that the effects observed in our initial experiment were BNST-specific. We then compared chemogenetic activation (hM3Dq) and inhibition (hM4Di) of BNST in a test of avoidance under extinction conditions. Because stimuli were delivered in an invariant way that was not contingent upon the subjects' response, this design allowed us to test all groups under common conditions. After AAV infusions and recovery, subjects received six days of avoidance training. 24 hours later, all subjects were exposed to 10 unreinforced CSs preceded by either CNO or vehicle. We found that CNO decreased avoidance in hM4Di subjects and increased avoidance in hM3Dq subjects relative to GFP and vehicle controls, confirming that BNST is not only necessary for normal levels of avoidance but is also sufficient to potentiate the response. These results demonstrate that BNST, a region known to mediate behavioral defense against low imminence threats, is a crucial substrate of avoidant behavior.

Disclosures: D.P. Guerra: None. **J.M. Moscarello:** None.

Poster

P660. Threat/Fear: Neural Circuits V

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Program #/Poster #: P660.02

Topic: G.01. Fear and Aversive Learning and Memory

Support: NARSAD Young Investigator Grant from Brain and Behavior Research Foundation Grant 25196

Title: A systems consolidation-like process recruits the retrosplenial cortex to the long-term maintenance of signaled avoidance

Authors: *M. R. KREITLOW[1], J. M. MOSCARELLO[1], L. J. KEPPLER[2];
[1]Texas A&M Univ., College Station, TX; [2]UT Hlth. San Antonio, San Antonio, TX

Abstract: The acquisition and expression of two-way signaled active avoidance (SAA) are supported by a changing neural substrate. Though recent work has begun to explore the circuitry underpinning the early stages of SAA, less is known about the mechanisms responsible for long-term maintenance of the response. Because of its established role in long-lasting forms of aversive memory, we hypothesized that the retrosplenial cortex (RSC) would be recruited to support SAA with extensive training. In our first experiment, male rats received intra-RSC injections of an AAV5 containing the gene construct for either the inhibitory hM4Di DREADD or GFP on a CamKII promoter. Following recovery, animals underwent an SAA training paradigm in which they learned to shuttle across a divided chamber during a tone in order to avoid foot shock. Animals were trained for 4 days to achieve asymptotic levels of avoidance. Subjects then received two SAA test sessions on days 5 and 6 that were preceded by counterbalanced IP injections of either CNO (3mg/kg) or VEH (saline + 10% DMSO). Animals received 2 more days of training, followed by another pair of test sessions on days 9 and 10. Though CNO inactivation of RSC had no effect on avoidance behavior after 4 days of training, RSC inhibition caused a robust decrement at the latter timepoint, demonstrating that long-term maintenance of the avoidance response requires RSC. We then set out to determine whether recruitment of the RSC was brought about by extensive training or whether time following initial acquisition would result in the RSC-dependence of SAA. hM4Di or GFP was expressed in RSC pyramidal neurons, as previously described. After recovery, subjects received either 4 days of training immediately followed by 2 SAA test sessions preceded by CNO or VEH, or 4 days of training followed by 4 days off in the homecage prior to 2 SAA test sessions. CNO inactivation of RSC attenuated avoidance behavior only in animals that received 4 days off in the homecage, suggesting that time following acquisition is sufficient to recruit RSC to SAA. These data confirm that RSC plays a role in the long-term maintenance of the avoidance response, and demonstrate that continued training beyond initial acquisition is not necessary for recruitment of RSC. To confirm the anatomical specificity of our results, we performed a control experiment in which we chemogenetically inactivated a neighboring region highly interconnected with RSC, the dorsal hippocampus, after 4 or 8 days of SAA. These manipulations had no effect on SAA. Collectively, our data demonstrate that RSC is a key substrate underlying the long-term maintenance of SAA and is recruited via a systems consolidation-like mechanism.

Disclosures: M.R. Kreitlow: None. **J.M. Moscarello:** None. **L.J. Keppler:** None.

Poster

P660. Threat/Fear: Neural Circuits V

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P660.03

Topic: G.01. Fear and Aversive Learning and Memory

Title: Expression of active avoidance in rats depends on the experience of a social partner that is present during conditioning.

Authors: *G. ERICKSON, A. SCOTT, T. MAZE, K. RODRIGUEZ, M. M. DIEHL; Psychological Sci., Kansas State Univ., Manhattan, KS

Abstract: Active avoidance of perceived danger is essential for survival. In rats, active avoidance can be studied under controlled laboratory settings using the platform-mediated avoidance (PMA) task, in which a rat learns to avoid a tone-signaled footshock by stepping onto a safe platform (Bravo-Rivera et al., 2014). Because PMA has only been studied in the absence of social cues, we were interested in whether prior PMA experience of a social partner would facilitate the learning and memory of avoidance in a naïve rat. We therefore modified the PMA task, in which two rats underwent PMA conditioning simultaneously, each with individual access to a food bar and platform. Male and female rats underwent PMA conditioning in the presence of a same-sex partner that was either naïve to the task or experienced (i.e. previously PMA-conditioned). The rats were separated by a perforated plexiglass barrier allowing for transmission of visual and olfactory cues. Across 10 days of conditioning, we measured percent time on platform and freezing to the tone. Preliminary analyses show no differences in avoidance or freezing between rats with an experienced (n = 6) vs. naïve partner (n = 11), nor were there differences in the number of shocks received (i.e., failures to successfully avoid), suggesting that the presence of a social partner does not affect PMA acquisition. After 10 days of social partner conditioning, rats underwent an additional conditioning session on Day 11 without their social partner. Preliminary data show that rats with an experienced partner (n = 6) spent more time avoiding compared to rats with a naïve partner (n = 3; experienced partner mean = 86% vs. naïve partner mean = 67%). Rats with an experienced partner also exhibited lower average freezing across all 9 tones compared to rats with a naïve partner (experienced partner mean = 34% average freezing vs. naïve partner mean = 49%). Together, these data suggest that the presence of an experienced partner during acquisition may improve performance of active avoidance when facing the same situation alone, but not affect performance during the learning period.

Disclosures: G. Erickson: None. **A. Scott:** None. **T. Maze:** None. **K. Rodriguez:** None. **M.M. Diehl:** None.

Poster

P660. Threat/Fear: Neural Circuits V

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Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P660.04

Topic: G.01. Fear and Aversive Learning and Memory

Support: UTSA Startup Funds

Title: Prefrontal function in threat and safety learning during normal and stress conditions, using a novel paradigm involving thermal stimuli

Authors: A. C. FELIX-ORTIZ[1], M. C. GARZA[3], D. T. ARRIAGA[1], D. T. HAJALI[1], S. M. LOPEZ[4], H.-G. LEE[2], *A. BURGOS-ROBLES[1];
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Abstract: Optimal learning of threat and safety is fundamental for animals to thrive in their environment. Growing evidence implicates the prefrontal cortex (PFC) in this ability during motivational conflict. However, it is still a matter of debate how distinct PFC divisions, such as the infralimbic (IL) and prelimbic (PL) cortices, contribute to the learning and coordination of behaviors associated to threat and safety during situations of conflict. Furthermore, it remains unclear how these PFC divisions contribute to alterations in threat and safety conflicts after prolonged psychological stress. In this study, we developed a novel task in mice using naturalistic thermal stimuli, cold (5°C) versus warm (30°C) zones in an acrylic apparatus, to simulate threat and safety, respectively. Discrete visual cues on the walls helped mice to distinguish the threat and safety zones during training. Mice were then required to remember these visual cues during a long-term memory test session while all zones had a uniform temperature. During the test session, mice avoided the previously cold zones, and spent most of the time in the previously warm zones. Mice in control groups that underwent training on an apparatus with a uniform temperature spent equal times in all zones during test. These findings indicate that mice can optimally distinguish, learn, and remember threat-like and safety-like areas of an environment using thermal stimuli. We next used a social isolation stress paradigm in which mice underwent isolated housing for 12 days, followed by 14 days of group housing prior to the thermal stimuli conflict task. This history of social isolation stress did not affect threat and safety detection during training in the thermal stimuli conflict task. However, stress produced a robust impairment in subsequent memory recall during the long-term test session. We finally tested the functional role of the IL and PL divisions of PFC using an ArchT-mediated optogenetic silencing strategy. Performance in the thermal stimuli conflict task during training was unaffected by either IL or PL silencing. However, IL silencing led to a robust impairment in memory recall in no-stress controls during the subsequent test session, thus mimicking the stress-related effect. In contrast, PL silencing during training fully prevented the memory recall impairment observed in stressed mice. Collectively, these findings suggest that the IL and PL divisions of PFC play important roles for both threat and safety learning associated to thermal stimuli, and that alterations in IL and PL activity play major contributions during stress-related alterations in threat and safety learning.

Disclosures: A.C. Felix-Ortiz: None. **M.C. Garza:** None. **D.T. Arriaga:** None. **D.T. Hajali:** None. **S.M. Lopez:** None. **H. Lee:** None. **A. Burgos-Robles:** None.

Poster

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Program #/Poster #: P660.05

Topic: G.01. Fear and Aversive Learning and Memory

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Whitehall Foundation Research Grant
DGSOM Seed Grant
Fay/Frank Seed Grant (Brain Research Foundation)
Klingenstein-Simons Fellowship
NSERC Postgraduate Fellowship

Title: Mapping the brain-wide axonal projection patterns of anatomically-defined prefrontal neurons

Authors: *M. W. GONGWER, C. KLUNE, R. CHEN, L. A. DENARDO;
Physiol., David Geffen Sch. of Med. at UCLA, Los Angeles, CA

Abstract: Descending projections from medial Prefrontal Cortex (mPFC) to its downstream targets are critical in the regulation of adaptive behaviors including decision making, social behavior and conditioned fear. Studies utilizing optogenetic and chemogenetic techniques have demonstrated that specific mPFC projections have distinct behavioral roles. It is known that long range projection neurons can innervate multiple target regions. However, the anatomical collateralization patterns of mPFC projection neurons is not well understood. Understanding the collateralization patterns of mPFC projections is necessary to understand how mPFC projections may simultaneously affect downstream brain regions to produce specific behaviors. In this study, we used a dual-viral strategy to label the brain-wide collateralization patterns of projections from mPFC to Nucleus Accumbens (NAc), Ventral Tegmental Area (VTA), and contralateral mPFC (cmPFC) in mice. An AAV expressing Cre recombinase was injected unilaterally into either VTA, NAc, or cmPFC. Then, a Cre-dependent AAV expressing a membrane-bound GFP fluorophore was injected unilaterally into mPFC. The membrane-bound nature of the GFP fluorophore allowed for the visualization of any axon collaterals innervating other target regions. Following six weeks of expression, brains were collected and cleared using the Adipoclear protocol. Light sheet fluorescence microscopy was used to image the entire left hemisphere of the intact brain. TRAILMAP, an axon classifier pipeline based on machine learning, was used to identify axons and quantify their density within brain regions. We observed that mPFC projections to NAc, VTA and cmPFC each display collateralization with multiple other brain areas. Our analyses revealed distinct collateralization patterns based on the projection target, with distinctions in cortical, amygdalar, thalamic, and hypothalamic axonal density. These data further our anatomical knowledge of mPFC projections and allow us to better understand how mPFC orchestrates behavior through its descending influence. The findings of this study can be

used to more accurately design and interpret experiments that manipulate neuronal projections to gain a more comprehensive understanding of mPFC function.

Disclosures: M.W. Gongwer: None. **C. Klune:** None. **R. Chen:** None. **L.A. DeNardo:** None.

Poster

P660. Threat/Fear: Neural Circuits V

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Program #/Poster #: P660.06

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIMH Grant K01MH116264
ARCS Foundation Fellowship
T32 Grant MH073526
Brain&Behavior Research Foundation
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UCLA DGSOM

Title: BehaviorDEPOT: a tool for automated behavior classification and analysis in rodents

Authors: *C. GABRIEL, Z. ZEIDLER, B. JIN, A. WU, L. A. DENARDO;
UCLA, Los Angeles, CA

Abstract: Quantitative descriptions of animal behavior are essential to understand the underlying neural substrates. Fear conditioning in rodents is a widely used assay that allows neuroscientists to probe the neural mechanisms of memory. To date, quantification of freezing behavior, a proxy for fear memory strength, is usually performed by hand or with expensive and inflexible commercial software. To overcome these barriers, we developed BehaviorDEPOT (DEcoding behavior based on POsitional Tracking), a MATLAB-based application containing six independent modules. The Experiment Module runs fear conditioning experiments using an Arduino-based design that interfaces with commercial hardware. The Analysis Module classifies freezing and analyzes spatiotemporal behavioral statistics in user-defined ways. The remaining modules can develop custom classifiers. Of note, the Inter-Rater Module establishes reliable 'ground-truth' human labels, making it broadly useful for scientists developing classifiers with any application. BehaviorDEPOT provides a simple, flexible, automated pipeline to move from pose tracking to reliably quantifying task-relevant behaviors.

Disclosures: C. Gabriel: None. **Z. Zeidler:** None. **B. Jin:** None. **A. Wu:** None. **L.A. DeNardo:** None.

Poster

P660. Threat/Fear: Neural Circuits V

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Program #/Poster #: P660.07

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIMH T32MH073526
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Brain and Behavior Foundation
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Brain Research Foundation
UCLA DGSOM

Title: Functional connectomes of amnesic and persistent memories

Authors: *B. JIN[1], A. WU[2], L. DENARDO[3];
[1]Molecular, Cellular, and Integrative Physiol., [2]Inst. for Society and Genet., [3]Dept. of Physiol., UCLA, Los Angeles, CA

Abstract: In infantile amnesia, memories formed early in life are short-lived, while those formed later can last a lifetime. We model this phenomenon in mice using contextual fear conditioning. Infant mice that undergo fear conditioning exhibit amnesia of fearful associations while adults exhibit long-lasting memories. To respond adaptively to fearful stimuli, the brain depends on the coordination of a broad network of cortical and subcortical brain regions. While the neural substrates underlying conditioned fear have been well-studied in the adult, the organization of fear memory networks in the developing brain remains poorly understood. We hypothesize that immature brain connectivity in development may cause infantile amnesia. Yet, whether and how the infant fear network undergoes reorganization or refinement across development to facilitate memory stability remains unclear. To address this issue, we performed whole brain mapping of activated neuronal populations using TRAP2 mice (Targeted Recombination in Active Populations) in combination with brain clearing and light sheet fluorescence microscopy. To identify neural signatures of memories that will become amnesic, we labeled neuronal ensembles activated by recent (1 day) fear memory at key developmental timepoints P17, 25, and 60 characterized by amnesic and lasting memories, respectively. Network analyses reveal that the functional organization of fear memories is dynamic across development. Importantly, our analyses suggest that there is increased cortical regulation of subcortical structures from adolescence to the young adult stage at P60. This age-dependent shift parallels the transition from shorter-lasting memories in infancy to longer-lasting memories in adolescent and adult mice, highlighting potential mechanisms that underlie the emergence of persistent fearful memories across development.

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Poster

P660. Threat/Fear: Neural Circuits V

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Program #/Poster #: P660.08

Topic: G.01. Fear and Aversive Learning and Memory

Support: NSERC Postgraduate Doctoral Award

Title: Development of medial prefrontal cortex circuitry and its role in the maturation of adaptive avoidance in mice

Authors: *C. KLUNE[1], C. GOODPASTER[1], R. CHEN[2], L. A. DENARDO[1];
[1]Physiol., [2]Computer Sci., UCLA, Los Angeles, CA

Abstract: The medial prefrontal cortex (mPFC) and its circuitry regulate numerous adaptive functions in both humans and rodents, including decision making, social behaviors, and mood. Many mPFC-dependent behaviors mature from adolescence to adulthood, which may reflect the protracted developmental period of mPFC. Despite the critical control that mPFC exerts over behavioral functions, when specific mPFC circuits mature and how they regulate behavior across development is poorly understood. This study aims to define the temporal development of mPFC projections to the nucleus accumbens (NAc) and basolateral amygdala (BLA) and their contribution to adaptive avoidance behavior from the postnatal period to adulthood in mice. A platform-mediated avoidance (PMA) task was modified to accommodate testing during the postnatal period. PMA, in which a fear-conditioned tone prompts rodents to navigate to a safety platform, has previously shown to be bidirectionally modulated by mPFC projections to NAc and BLA in rats. In a single training session, adult mice learned to avoid the shock by entering the platform upon presentation of the tone. The next day, avoidance memory was tested in a retrieval session during which tones were presented without the shock. Adult mice spent more time on the platform during the tone periods than is expected by chance, suggesting retention of the learned avoidance response. Our data demonstrate that adaptive responding in this task is developmentally regulated. At postnatal day (P)17, mice do not learn to avoid the shock. By P23, mice learn to avoid the shock during training but do not show robust levels of avoidance during retrieval. At P28, mice learn to avoid and demonstrate retention of learned avoidance 1 day later. Developmental viral tracing data reveals that mPFC axon density in both NAc and BLA increases from P16 to P28 and declines from P28 to adulthood. These changes in axonal innervation density may reflect arborization and pruning events. Finally, optogenetics was used to assess the functional role of mPFC projections to NAc and BLA during PMA at the developmental timepoints. In both adulthood and at P28, optogenetic activation of mPFC to NAc projections during the tone period of the retrieval session significantly decreased time on the platform. Excitation of mPFC to BLA neurons is expected to augment avoidance in adults and

may be able to increase avoidance in P23 mice. The findings of this study will elucidate how mPFC projections develop and contribute to the emergence of adaptive behavior. Understanding how mPFC circuits develop in the typical brain is foundational to how their development may be perturbed and contribute to disease states.

Disclosures: C. Klune: None. **C. Goodpaster:** None. **R. Chen:** None. **L.A. DeNardo:** None.

Poster

P660. Threat/Fear: Neural Circuits V

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Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P660.09

Topic: G.01. Fear and Aversive Learning and Memory

Support: College of Liberal Arts, Texas A&M University

Title: The neurosteroid 3alpha-androstanediol does not confer state dependence to contextual fear in male rats

Authors: *R. BLAIR[1], N. NAGAYA[2];
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Abstract: Sex steroids and their neuroactive metabolites can modulate emotional behavior in both humans and rodents. We have previously shown that allopregnanolone (ALLO), a progesterone metabolite, can confer state dependence to contextual fear when infused into the bed nucleus of the stria terminalis (BNST) of adult male rats. The testosterone metabolite, 3α-androstanediol (3α-diol), is similar to ALLO such that it produces anxiolysis and acts as a positive allosteric modulator of the $GABA_A$ receptor. In the present study, we hypothesized that 3α-diol, like ALLO, may confer state dependence to contextual fear via modulation of GABAergic tone. To this end, we bilaterally infused 3α-diol (7.3 μg/μL) or vehicle (VEH; 30% 2-hydroxypropyl-β-cyclodextrin) into the BNST of adult male rats (n = 40) 10 min prior to Pavlovian fear conditioning with 5 tone (CS; 2 kHz, 10 s, 80 dB)-footshock (2 s, 1 mA) pairings. On subsequent days, fear retention was tested by separate exposures to context (10 min) and cue (4 CS-alone trials in a novel context) following infusion of either the same or different drug (3α-diol or VEH). Acquisition and expression of contextual and cued fear were similar for all animals, regardless of drug treatment. Further, no significant interaction was found between drug infused on conditioning and testing days, suggesting that 3α-diol does not confer state dependence to either contextual or cued fear. A subset of animals (n = 15) was subjected to extinction training and testing sessions consisting of 45 CS-only presentations each in a novel context. After infusion of either 3α-diol or VEH, rats received extinction training followed by an extinction retention test 24 h later. Acquisition of extinction differed between drug treatment groups. Compared to VEH-treated animals, those treated with 3α-diol showed enhanced cued

fear extinction, especially over the first 20 trials. Extinction retention did not differ between treatment groups. Overall, these findings suggest that, unlike the neurosteroid ALLO, 3α-diol does not confer state dependence to contextual fear via the BNST but may modulate cued fear extinction.

Disclosures: R. Blair: None. **N. Nagaya:** None.

Poster

P660. Threat/Fear: Neural Circuits V

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Program #/Poster #: P660.10

Topic: G.01. Fear and Aversive Learning and Memory

Support: K08 MH014743-01A1
T-32-NS076401

Title: Maternal presence during threat learning modulates functional connectivity and community structure in infant rats

Authors: *A. M. WHITE[1,2,3], J. DEBIEC[1,2,3];
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Abstract: As infant rats begin to develop independence from their mother, their nervous system must start responding to and learning about threats. However, it remains ecologically relevant for threat learning to be under the control of the mother until the infant has matured to full independence. Previous studies have shown that pups in the second week of life that undergo Pavlovian odor-shock conditioning in the presence of an anesthetized mother will not demonstrate threat responses to future presentations of the conditioned odor. This is mediated in part by the mother's ability to suppress amygdala activity during threat learning. There has been a growing appreciation that patterns of neuronal activity between groups of brain areas, rather than simple absolute increases or decreases in activity, are essential for this type of learning. We used a graph theoretical approach to analyze patterns of immediate early gene expression and investigate differences in functional networks engaged by postnatal day 12-15 male and female infant rats that underwent Pavlovian odor-shock conditioning without or with maternal presence. Functional connectivity between regions of interest previously shown to be critical for threat learning and processing of social cues shifted depending on whether the mother was present during threat learning. A community detection algorithm revealed that maternal presence during threat learning altered network structure and which regions function as hubs. Our results demonstrate the power of the graph theoretical approach and the power of the mother's ability to modulate coordinated neural activity within her pups during a critical developmental timepoint.

Disclosures: A.M. White: None. **J. Debiec:** None.

Poster

P660. Threat/Fear: Neural Circuits V

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Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P660.11

Topic: G.01. Fear and Aversive Learning and Memory

Support: UTSA Startup Funds

Title: New insights on the role of distinct prefrontal cortical divisions using a novel task for active avoidance learning that involves a dynamic option for safety.

Authors: *M. GARZA;
Univ. of Texas At San Antonio Dept. of Biology/Neurobiology, San Antonio, TX

Abstract: Active avoidance is a learned, complex, adaptive behavior that helps animals prevent harm associated with imminent threat. Although previous studies have implicated the prefrontal cortex (PFC) in active avoidance, the functional role for distinct PFC divisions - such as the infralimbic cortex (IL), prelimbic cortex (PL), and anterior cingulate cortex (ACC) - still remain unclear. To address this, we developed a novel variation of the platform-mediated avoidance paradigm (Diehl, Bravo-Rivera, and Quirk 2019) that isolates threat-related behaviors and active avoidance responses while eliminating appetitive reward components and motivational conflict. In this new task, mice received auditory cues (80 dB, 20 sec) that predicted mild electric shocks (0.30 mA, 2 sec, co-terminating with cues). As mice learned the tone-shock association (20 trials per session), they also learned to avoid shocks by stepping onto a motorized, non-electrified dynamic platform that was inserted into one corner of the apparatus before the beginning of each trial, and retracted shortly after the termination of each trial. In this way, the platform was only available when mice needed it to perform active avoidance responses and minimized passive avoidance responses (i.e., staying on the platform during the inter-trial intervals). Results showed that mice initially exhibited conditioned freezing responses that gradually transitioned into active avoidance responses. We next implemented an ArchT-mediated optogenetic inhibition approach to silence glutamatergic outputs from the distinct PFC subregions and tested their roles during the new avoidance task. These experiments revealed that selective silencing of the IL-PFC glutamatergic outputs significantly impaired the gradual decay of conditioned freezing and strongly affected the acquisition of active avoidance responses. Selective silencing of the PL-PFC glutamatergic outputs produced no significant effects on freezing or avoidance behaviors. Current experiments are testing the role of the ACC-PFC in this task. Therefore, while previous studies suggest crucial functional roles for the distinct PFC divisions in active avoidance, new studies should consider that integration of appetitive components and motivational conflict could plausibly confound findings on active avoidance learning.

Disclosures: M. Garza: None.

Poster

P729. Molecular Mechanisms of Addictive Drugs

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Program #/Poster #: P729.01

Topic: G.09. Drugs of Abuse and Addiction

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NIH Grant AA025113
NIH Grant DA039168
NIH Grant U01DA050243

Title: Evidence of the potential therapeutic utility of a selective PDE4B inhibitor for reducing methamphetamine-taking in two substrains of C57BL/6 mice

Authors: *K. M. HONEYWELL[1], E. VAN DOREN[2], K. K. SZUMLINSKI[1];
[2]Psychological and Brain Sci., [1]Univ. of California, Santa Barbara, Santa Barbara, CA

Abstract: Tolerability issues accompanying the targeting of neuroinflammation using inhibitors of the cAMP-hydrolyzing enzyme, phosphodiesterase 4 (PDE4), may limit therapeutic utility for reducing drug-taking and -craving. Herein, we examined the effects of a PDE4B-selective inhibitor, A33, on methamphetamine (MA) intake by female and male mice of genetically distinct C57BL/6J (B6J) and C57BL/6NJ (B6NJ) substrains that differ both in their behavioral sensitivity to MA and their innate immune response. Mice were first trained to acquire oral MA self-administration (100 mg/L) under operant-conditioning procedures. Then, the dose-response function (0-1.0 mg/kg) for A33's effects upon the maintenance of oral MA intake was determined. Females and B6J mice more readily acquired MA self-administration and self-administered more MA than males and B6NJ mice. Irrespective of sex or substrain, A33 pretreatment reduced the intake of 100 mg/L MA. We next compared the dose- and demand-response functions for oral MA intake and detected no sex or substrain differences in MA reinforcement or intake. Finally, mice were pretreated with either 0 or 1.0 mg/kg A33 prior to tests for cue-reinforced responding under extinction conditions. However, we failed to detect any significant effect of A33 pretreatment on cue-reinforced responding, nor did we detect significant sex or substrain differences. These data provide the first evidence that selective PDE4B inhibition is an effective strategy for reducing MA reinforcement, but not MA cue-reactivity, in mice. A33 was equally effective in males and females and exerts a quantitatively similar reduction in MA intake in mice with a genetic predisposition for high versus moderate behavioral sensitivity to MA. Such findings support the potential clinical utility of selectively targeting PDE4B for reducing active MA-taking in individuals with MA Use Disorder.

Disclosures: K.M. Honeywell: None. **E. Van Doren:** None. **K.K. Szumlinski:** None.

Poster

P729. Molecular Mechanisms of Addictive Drugs

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Program #/Poster #: P729.02

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant AA024044
NIH Grant HD052731
Bill and Melinda Gates Foundation
Brain Research Foundation

Title: Camkii-dependent homer2 phosphorylation does not alter alcohol reinforcement in mature adult or middle-aged mice.

Authors: *F. J. CANO[1], S. L. SCUDDER[1], M. DOOR[1], K. M. HUBER[2], K. K. SZUMLINSKI[1];
[1]Univ. California-Santa Barbara, Santa Barbara, CA; [2]Neurosci., Univ. of Texas Southwestern Med. Ctr. at Dallas, Dallas, TX

Abstract: Homer2 is a glutamate receptor-related post-synaptic scaffolding protein that is up-regulated throughout the extended amygdala, affecting both alcohol intake and intoxication. Homer2 is phosphorylated on S117/S216 by calcium-calmodulin kinase II alpha (CaMKII) and this phosphorylation causes a rapid dissociation of mGluR5-Homer2 binding. congenic C57BL/6J mice with alanine point mutations at S117/216 that prevent Homer2 phosphorylation (AA mice) exhibit blunted binge alcohol-drinking, but do not differ from wild-type (WT) littermates in terms of alcohol consumption under continuous-access procedures. Herein, we compared alcohol reinforcement between AA and WT mice. Female and male mice were trained for 2 weeks (Cohort 1; mice approximately 12 months old) or 4 weeks (Cohort 2; mice approximately 6 months old) to nose-poke for 20% alcohol under an FR1 schedule of reinforcement during daily 30-min sessions. During early acquisition training, AA mice from the older Cohort 1 exhibited significantly lower responding and consumed significantly less 20% alcohol than WT animals, with strong trends detected also for lower reinforced responding and reinforcers earned. However, this genotypic difference in early alcohol responding did not replicate in the younger Cohort 2 animals. No genotypic differences were observed in either cohort for the proportion of mice meeting the acquisition criteria or for the alcohol dose- and demand-response functions, although a sex difference in responding was detected in Cohort 2 that was not apparent in the older mice. Taken together, the present data do not support a key role for Homer2 phosphorylation in alcohol reinforcement or intake under operant-conditioning procedures. Such findings further the notion that CaMKII alpha-dependent phosphorylation of

Homer2 plays a relatively selective role in regulating binge-drinking, which likely reflects dynamical interactions between Homer2 and mGlu5 within the extended amygdala. Funding provided by NIH grants AA024044 to KKS and HD052731 to KMH, as well as a Brain Research Foundation Early Investigator award to SLS and a Gates Foundation Predoctoral Fellowship to FJC.

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Poster

P729. Molecular Mechanisms of Addictive Drugs

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Program #/Poster #: P729.03

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant DA053328

Title: Effects of inhibiting AMPA and NMDA-type glutamate receptors in the ventromedial prefrontal cortex on sucrose self-administration

Authors: *L. L. HUERTA SANCHEZ, M. LANZ, K. K. SZUMLINSKI;
Univ. of California, Santa Barbara, Santa Barbara, CA

Abstract: The glutamatergic system is purported to play an important role in the self-administration of both drug and non-drug reinforcers, such as sucrose. However, sucrose-experienced rats exhibit blunted glutamate release within the ventromedial prefrontal cortex (vmPFC), calling to question the role for glutamate receptor activation within this region in driving sucrose reinforcement. To address this question, this study investigated the role of vmPFC AMPA- and NMDA -type glutamate receptors in sucrose-taking. For this, male Sprague-Dawley rats were implanted with bilateral guide cannulae aimed over the vmPFC and were trained to self-administer sucrose pellets (6hr/day) for 10 days . Using a within-subjects design, rats were microinjected (0.5 µl per side) with the GluA2-lacking selective AMPA antagonist Naspm (40 µg), the non-selective AMPA receptor antagonist NBQX (1 µg), the GluN2A-selective NMDA antagonist PPPA (1.65 nmol), the GluN2B-selective NMDA antagonist TCN-237 (10 µM) and the non-selective NMDA receptor antagonist D-AP5 (2.5 µg). Following each microinjection, rats underwent a 2-hr test for sucrose self-administration. Microinjection of D-AP5 increased responding for sucrose reinforcement, while no significant effects were observed for the other compounds tested. These results indicate that activation of NMDA receptors within the vmPFC suppress sucrose self-administration, whereas AMPA receptors do not appear to be involved.

Disclosures: L.L. Huerta Sanchez: None. **M. Lanz:** None. **K.K. Szumlinski:** None.

Poster

P729. Molecular Mechanisms of Addictive Drugs

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P729.04

Topic: G.09. Drugs of Abuse and Addiction

Support: Ministerio de Ciencia e Innovación, Gobierno de España PID2019-109390RBI00

Title: Expression of immediate early genes after an acute administration of novel synthetic cathinones in mice.

Authors: *E. URQUIZU[1,2], M. ESPINOSA-VELASCO[2], N. NADAL-GRATACOS[1,2,3], P. PUIGSESLLOSES[2], M. BUENROSTRO-JÁUREGUI[1], X. BATLLORI[3], X. BERZOSA[3], D. PUBILL[2], J. CAMARASA[2], E. ESCUBEDO[2], R. LÓPEZ-ARNAU[2];
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Abstract: Synthetic cathinones, one of the most prevalent group of new psychoactive substances, have become a global phenomenon because of their easy accessibility and similar effects to cocaine or MDMA. It is known that continuous drug exposure causes neuroadaptations in dopaminergic neurons of several brain regions, which are thought to be involved in the transition from recreational drug use to drug addiction. However, these changes begin with variations on immediate early genes (IEGs), underlying the addictive potential of these drugs. Thus, the aim of this project was to study the changes in IEGs expression involved in the addictive process after an acute administration of novel synthetic cathinones: pentedrone, pentylone, N-ethyl-pentedrone (NEPD), N-ethyl pentylone (NEP), 4-methyl pentedrone (4-MPD) and 4-methyl--ethylaminopentedrone (4-MeAP). CD-1 male mice were injected the corresponding synthetic cathinone (10 mg/kg, i.p.) or saline solution. RNA was extracted from ventral striatum (VS), dorsal striatum (DS) and medial prefrontal cortex (mPFC) in order to analyze the expression of the IEGs *Arc*, *c-fos* and *bdnf 30* or 120 min after administration by a quantitative RT- PCR technique. Results revealed that an acute injection of pentylone, NEPD, NEP and 4-MeAP induced a significant increase of *Arc* gene expression in VS and DS 120 min after administration. However, only NEP significantly increased mRNA levels in VS 30 min after injection. All the synthetic cathinones tested in this study induced a significant increase in *c-fos* expression in DS 30 and/or 120 min post-administration, except for 4-MPD. Regarding VS, only NEPD, NEP and pentedrone administrations were able to significantly increase *c-fos* levels. Although a tendency to increase *bdnf* expression was observed after an acute administration of all compounds tested, only NEP-treated mice showed a significant overexpression of mRNA

bdnf levels. This study evidences that novel synthetic cathinones are able to up-regulate the expression of some IEGs in mouse DS, VS and mPFC. This overexpression may lead to prolonged neuroadaptations, memory formation, neuroplasticity and in changes in behavior to psychostimulants. These modifications could lead to cellular cascades and be a sign of the early stage of drug addiction.

Disclosures: E. Urquizu: None. **M. Espinosa-Velasco:** None. **N. Nadal-Gratacos:** None. **P. Puigseslloses:** None. **M. Buenrostro-Jáuregui:** None. **X. Batllori:** None. **X. Berzosa:** None. **D. Pubill:** None. **J. Camarasa:** None. **E. Escubedo:** None. **R. López-Arnau:** None.

Poster

P729. Molecular Mechanisms of Addictive Drugs

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P729.05

Topic: G.09. Drugs of Abuse and Addiction

Title: Distinct DNA methylation signatures in mouse nucleus accumbens in alcohol use disorder susceptibility.

Authors: *G. KAPLAN[1], Y. LI[1], R. S. JASROTIA[1], J. CHITAMAN[1], N. WADDELL[1], K. ABREU[1], H. XU[1], R. HEDINGER[1], C. REMEDIES[1], G. XU[2], J. FENG[1];
[1]Biol. Sci., Florida State Univ., Tallahassee, FL; [2]Inst. of Biochem. and Cell Biology, Chinese Acad. of Sci., Shanghai, China

Abstract: Most adults consume some alcohol, yet only around 10% of them develop an alcohol use disorder (AUD). Elucidating the neural mechanisms underlying susceptibility to AUD represents an expeditious approach to relieving the alcohol disease burden. Dysregulation of DNA methylation, a major epigenetic mechanism, is increasingly implicated in the etiology of AUD. In this study, we utilized a mouse model of alcohol behavioral sensitization - a form of behavioral plasticity thought to underlie the transition from recreational to compulsive drug use - to explore the DNA epigenetic underpinnings of alcohol use disorder susceptibility differences. Adult male C57BL6/J mice were given daily i.p. injections of alcohol or saline. Based on their locomotor response on day 21, mice from the top and bottom quartiles of the alcohol-treated group were classified as sensitized or non-sensitized, respectively. In subsequent self-administration experiments, sensitized mice consumed significantly more alcohol, but not sucrose, demonstrating relevance to AUD susceptibility. We then profiled whole-genome DNA methylation in the nucleus accumbens (NAc) using enzymatic methylation sequencing (EM-seq). We identified substantial DNA methylation differences between sensitized and non-sensitized mice enriched in addiction related gene categories. Differential methylation was also associated with phenotype-specific alterations in chromatin accessibility and gene expression. Furthermore, we found DNA methylcytosine dioxygenase *Tet3* was differentially expressed between

sensitized and non-sensitized mice. Knocking down *Tet3* in the NAc of alcohol-naïve mice attenuated alcohol sensitization and recapitulated some DNA methylation changes associated with AUD. Our study therefore demonstrates that behavioral sensitization differences to alcohol are associated with distinct DNA methylation signatures in mouse nucleus accumbens, which supports a functional role of DNA methylation in AUD susceptibility.

Disclosures: G. Kaplan: None. **Y. Li:** None. **R.S. Jasrotia:** None. **J. Chitaman:** None. **N. Waddell:** None. **K. Abreu:** None. **H. Xu:** None. **R. Hedinger:** None. **C. Remedies:** None. **G. Xu:** None. **J. Feng:** None.

Poster

P729. Molecular Mechanisms of Addictive Drugs

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Program #/Poster #: P729.06

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant R00DA045795
NIDA Grant P30DA033934
NIH-NCI Grant P30 CA016059

Title: Reprogramming ZFP189 transcription factor function within the nucleus accumbens regulates drug specific behaviors and transcription

Authors: *J. A. PICONE[1], G. SILVA[2], X. CUI[1], P. J. HAMILTON[1];
[1]Anat. and Neurobio., [2]Pharmacol. and Toxicology, Virginia Commonwealth Univ., Richmond, VA

Abstract: Understanding the molecular drivers of the stages of drug addiction may allow for the design of pharmacotherapies that block or reverse key events of the progression of drug addiction. *Zfp189* is a CREB-target gene which itself encodes an unstudied nucleus accumbens (NAc) neuronal transcription factor that has been demonstrated to regulate transcriptional networks in neuropsychiatric disorders. In rodents, *Zfp189* gene expression increases following exposure to psychostimulants, like cocaine. To further examine the downstream relationship between ZFP189 and physiological response to different classes of drugs of abuse, three re-engineered ZFP189 variants were designed to interrogate ZFP189-mediated gene regulation and subsequent behaviors following exposure to psychostimulants (cocaine) or opiates (morphine). The three ZFP189 variants used were: $ZFP189^{WT}$ (the endogenous gene with transcriptionally inhibitory KRAB domain), $ZFP189^{DN}$ (maintains binding domain but lacks KRAB domain), and $ZFP189^{VPR}$ (KRAB domain was replaced with the transcriptional activator VP64-p65-Rta (VPR)). A luciferase reporter gene possessing ZFP189 response element illuminated that $ZFP189^{WT}$ functions as gene repressor and $ZFP189^{VPR}$ exerts opposite function as a robust gene

activator. The nucleus accumbens of male and female C57/BL6J mice were transduced with ZFP189 variant constructs via viral-mediated gene transfer. We then performed a drug locomotor sensitization assay for repeated injections of saline, cocaine, or morphine. Mice expressing $ZFP189^{VPR}$ exhibit heightened cocaine locomotor sensitization relative to $ZFP189^{WT}$ and $ZFP189^{DN}$. Interestingly, this increased locomotion is unique to cocaine, as there is no difference in locomotor activity between the ZFP189 variant groups in response to saline or morphine administration. Microdissecting virally manipulated NAc and performing RNA sequencing revealed striking differences in the ability of $ZFP189^{VPR}$ to regulate endogenous gene expression, with cocaine treated animals possessing far more differentially regulated genes (DEGs) than similarly manipulated morphine treated mice. These results suggest a drug-specific role for the function of ZFP189 in driving brain transcriptional and behavioral responses to cocaine, but not morphine.

Disclosures: J.A. Picone: None. **G. Silva:** None. **X. Cui:** None. **P.J. Hamilton:** None.

Poster

P729. Molecular Mechanisms of Addictive Drugs

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Program #/Poster #: P729.07

Topic: G.09. Drugs of Abuse and Addiction

Support: 5R01DA046818-02

Title: Histone demethylase JMJD3 mediates opioid-induced cellular and behavioral plasticity

Authors: *S. MITRA[1], P. H. GOBIRA[2], C. T. WERNER[1], J. A. MARTIN[1], S. THOMAS[1], C. AN[1], M. IIDA[1], S. SWENSON[1], R. CHANDRA[3], R. L. NEVE[4], M. LOBO[5], D. M. DIETZ[6]; [1]Dept. of Pharmacol. & Toxicology, State Univ. of New York at Buffalo, Buffalo, NY; [2]Univ. of São Paulo, Ribeirao Preto, Brazil; [3]Anat. and Neurobio., Univ. of Maryland Baltimore, Baltimore, MD; [4]Neurol., Massachusetts Gen. Hosp., Cambridge, MA; [5]Anat. and Neurobio., Univ. of Maryland Sch. of Med., Baltimore, MD; [6]State Univ. of New York, Buffalo, NY

Abstract: Opioid use disorder is a chronic and debilitating disease that is marked by relapse after periods of abstinence. A multitude of drug-elicited neuroadaptations in brain regions governing reward such as the Nucleus Accumbens (NAc), mediate maladaptive craving behaviors leading to relapse. One of the persistent changes underlying neuroadaptive mechanisms during abstinence from drugs of abuse is epigenetic modifications of DNA and histones. Here we show that following prolonged abstinence (AD14) from heroin self-administration, histone demethylase, JMJD3 is increased in the NAc. Further, we demonstrate that this increase is specific to D2+ MSNs, demonstrating a cell-type-specific regulation. To demonstrate the functional significance of these changes, we employed both pharmacological

and viral-mediated approaches to modulate JMJD3 expression in the NAc, which demonstrated that JMJD3 levels/activity are sufficient to regulate cue-induced heroin seeking. Finally, we present data that the epigenetic factors are themselves regulated through the TGF-β super-family which we have previously demonstrated to be essential for drug-seeking behaviors. Together these data highlight that JMJD3 is essential in mediating persistent cellular and behavioral adaptations following heroin exposure.

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Poster

P729. Molecular Mechanisms of Addictive Drugs

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH/NIDA R01DA047843
Binational Science Foundation #2017252

Title: Molecular Profiling of the Ventral Pallidum in Mice

Authors: *R. CAMPBELL[1], H. NAM[2], M. BASU[1], M. ENGELN[3], R. CHANDRA[3], S. A. AMENT[4], M. LOBO[5];
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Abstract: The ventral pallidum (VP) is a critical brain region for drug-seeking behaviors, as it is a primary output to the several regions within the reward system. Further, this region is unique because it receives dense input from both ventral striatal (nucleus accumbens) projection neuron subtypes compared to other brain regions that display more segregated input. Recent circuitry mapping revealed that distinct VP cell types and projections regulate specific behavioral responses to rewarding and aversive stimuli, including drugs of abuse. However, a comprehensive molecular characterization of these cell types and circuits have not been performed. Here, we generated several sequencing data sets to examine the molecular architecture of the VP. First, using Cre-dependent retrograde labeling and Ribotag-transgenic mice, we characterized the translatome of distinct mouse VP-projection cells. Retrograde Cre virus was infused into either the Lateral Hypothalamus (LH), Ventral Tegmental Area (VTA), Lateral Habenula (LHb) or Medial Dorsal Thalamus (MDT) of floxed-Ribotag male mice. Following viral expression, ventral pallidum tissue was extracted and ribosome-associated

mRNA was isolated to prepare libraries for RNA-sequencing. From our bioinformatic analyses, we identified genes and gene networks enriched within a specific VP projection cell-type. Additionally, using gene ontology analysis, we detected biological processes specific to each VP-projection cell type. Currently, we are performing single-nucleus RNA-sequencing on VP tissue from male and female mice. Through subclustering analysis, we will identify several distinct VP neuronal populations and new marker genes for each transcriptionally distinct population. Altogether, these investigations aim to detect the cellular populations present within the VP and identify novel gene makers to study in VP function and drug use disorders.

Disclosures: R. Campbell: None. **H. Nam:** None. **M. Basu:** None. **M. Engeln:** None. **R. Chandra:** None. **S.A. Ament:** None. **M. Lobo:** None.

Poster

P729. Molecular Mechanisms of Addictive Drugs

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Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P729.09

Topic: G.09. Drugs of Abuse and Addiction

Title: Using immediate early gene expression to characterize properties of behavioral ensembles in the nucleus accumbens

Authors: *A. MASKERI[1], D. A. BURKE[2], V. A. ALVAREZ[3];
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Abstract: The nucleus accumbens (NAc) functions as a “central hub” of the brain reward pathway and is a critical site of dysfunction in cases of substance use disorders. The primary cell type of this region is the projection medium spiny neuron (MSN), which has two subtypes: D1 and D2 MSNs, with opposing roles in driving motivated behavior. The NAc is generally thought to function as a collection of ensembles -- sparsely distributed neurons that drive specific NAc-dependent behaviors. To better understand the properties of these ensembles, we first characterized behaviors and methods for identifying and manipulating ensembles associated with responses to rewarding and aversive stimuli. Mice expressing a tamoxifen inducible Cre under the promotor for the immediate early gene Fos (TRAP2 mice) were used to label NAc ensembles underlying cocaine-sensitized locomotion. After quantifying the proportions of each MSN subtype, we found them to be present in approximately equal amounts within the ensemble. However, only the D2 MSNs were shown to correlate with locomotion. Current experiments are aimed at characterizing TRAP2 ensembles in the NAc during the expression of aversive learning, specifically foot shock-conditioned freezing. The goal for future experiments is to compare the neuronal overlap and exclusivity of ensembles underlying opposing behaviors, as well as to

measure the synaptic connectivity between and among distinct ensembles. Understanding the properties of these ensembles will ultimately lead us to a better understanding of motivated behavior and substance use disorders.

Disclosures: A. Maskeri: None. **D.A. Burke:** None. **V.A. Alvarez:** None.

Poster

P734. Learning and Memory in Addiction II

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Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P734.01

Topic: G.09. Drugs of Abuse and Addiction

Support: Natural Sciences and Engineering Research Council of Canada

Title: Extinction of conditioned memory modulation

Authors: *T. FRANCIS, F. LERI;
Psychology, Univ. of Guelph, Guelph, ON, Canada

Abstract: It has been established that exposure to drug conditioned stimuli (CS) can enhance memory consolidation, indicating that drug CSs not only produce changes in behaviour, but can also impact cognition. Therefore, it is possible that conditioned memory modulation, like other conditioned responses, will display extinction. The current study used classical and operant conditioning techniques to test this hypothesis in male Sprague-Dawley rats performing an object location (OL) memory task. To establish the CS in Experiment 1, rats were injected with heroin (0, 0.3, 1 mg/kg, S.C.) and immediately placed in operant chambers for 1 hr, once a day for 5 consecutive days. It was found that exposure to the heroin-CS four days after conditioning dose-dependently enhanced object location memory. In addition, when tested after 6 exposures to the CS without receiving heroin, the CSs ability to modulate memory was significantly reduced. In Experiment 2, rats were trained to intravenously self-administer heroin (0.05 mg/kg/inf) on a continuous reinforcement schedule for 12 days. It was found that exposure to the heroin-CS four days after training also enhanced object location memory, and when tested after 6 exposures to the CS without receiving heroin, the CSs ability to modulate memory was attenuated. Taken together, these data indicate that conditioned memory modulation, similarly to other conditioned drug effects, displays both pavlovian and instrumental extinction.

Disclosures: T. Francis: None. **F. Leri:** None.

Poster

P734. Learning and Memory in Addiction II

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Program #/Poster #: P734.02

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant KL2 TR001862
NIH Grant K01 AA027832

Title: Biased memories for different components of alcohol-related events

Authors: *B. B. HARRIS, E. V. GOLDFARB;
Dept. of Psychiatry, Yale Sch. of Med., New Haven, CT

Abstract: Memory plays a key role in addiction development and relapse. Crucially, memories of a single event can be broken down into items (individual parts of a memory) and contexts (integrations of different elements of a memory). Recent work indicates that individuals with alcohol use disorder are biased to remember precise alcohol-related contexts, while disproportionately forgetting items. However, the neural mechanisms underlying the formation of distinct components of alcohol-related memories are unclear. We hypothesize that alcohol-related events will be remembered differently from neutral events in social drinkers, and that alcohol-related memories will be associated with stronger BOLD responses in the dorsolateral striatum and perirhinal cortex (items) and hippocampus (contexts). We first developed and validated a set of 320 matched alcohol-related and neutral handheld object stimuli for use in a two-day memory task. Next, we enrolled 8 participants (mean age = 29.25, 63% female, AUDIT = 5.50, target N = 28) in an ongoing fMRI experiment. During the study, participants encoded 160 associations between pairs of objects (50% alcohol, 50% neutral) and neutral scenes. 24 hours later, memories for items (object recognition), contexts (cued recognition of scenes associated with objects) and affect were assessed, followed by an Alcohol Taste Test. Preliminary analyses show that, as expected, alcohol stimuli elicited stronger craving and affective responses at encoding ($p = .01$). After 24 hours, participants showed a slight memory bias for neutral items ($p = .11$) and "gist" memory for alcohol-related contexts ($p < .01$). Interestingly, preliminary evidence demonstrates that these memory biases (particularly item memory) predicted subsequent alcohol consumption ($p < .05$). Ongoing subsequent memory analyses are leveraging fMRI data to identify regions in which BOLD signal at encoding supports successful memory formation for alcohol-related items and contexts (i.e., remembered vs forgotten for alcohol vs neutral). This research has the potential to uncover the neural mechanisms supporting memories associated with addiction and may help guide treatment. Future research should strive to elucidate these mechanisms in clinical and non-drinking participant populations.

Disclosures: B.B. Harris: None. **E.V. Goldfarb:** None.

Poster

P734. Learning and Memory in Addiction II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P734.03

Topic: G.09. Drugs of Abuse and Addiction

Title: Contribution of impulsivity and contextual cues to the addictive effects of caffeine

Authors: J. CEBRIAN[1], ***G. GONZALEZ-CUEVAS**[2];
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Abstract: Caffeine is currently the most widely used psychoactive drug worldwide. Although, as such, it has the potential for developing addiction, the implications of caffeine use in addictive processes such as impulsivity and contextual cues' induced craving are still largely unknown. The present investigation, consisting of a correlational study and two experiments, aimed at studying these associations and to manipulate experimentally both caffeine acute consumption (and its expectancies) as well coffee-related cues to clarify the effects of caffeine on impulsivity and craving. Our sample comprised a total of 201 participants counting all three studies. Our results indicated that caffeine consumption was positively associated with higher levels of impulsive behavior as well with an enhancement of cue-induced coffee craving. Even though the manipulation of caffeine acute consumption and its expectancies did not alter impulsive behavior, the contextual cue associated with coffee produced increased perceived activation together with a deficit in response inhibition. All in all, these findings suggest that a lack of impulse control linked to contextual cues may be key to the addictive effects of caffeine. Nonetheless, future research is warranted to continue unraveling the causal role of impulsivity and contextual cues in the development of caffeine-related addiction, especially in relation to poly-drug use.

Disclosures: J. Cebrian: None. **G. Gonzalez-Cuevas:** None.

Poster

P734. Learning and Memory in Addiction II

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Program #/Poster #: P734.04

Topic: G.09. Drugs of Abuse and Addiction

Support: Canadian Biomarker Integration Network in Depression, Ontario Brain Institute

Title: Conditioned antidepressant effects of ketamine: a comparison to bupropion and escitalopram

Authors: B. MELANSON[1], M. WOLTER[1], Z. LEATHAM[1], T. D. LAPOINTE[1], *F. LERI[2]; [1]Univ. of Guelph, Guelph, ON, Canada; [2]Univ. Guelph, Guelph, ON, Canada

Abstract: The rapid and long-lasting antidepressant effects of ketamine (KET) set this drug apart from clinically established antidepressants like the selective serotonin reuptake inhibitor escitalopram (ESC) and the norepinephrine-dopamine reuptake inhibitor bupropion (BUP). There are suggestions from studies in animals that the antidepressant effect of KET may involve a drug-environment interaction, whereby a context paired with a psychoactive drug acquires the ability to influence behavior. Thus, in Experiment 1, male Sprague-Dawley rats were conditioned with 0.9% saline (w/v; SAL) in a vehicle-paired context (CS-), and with 10 or 20 mg/kg KET, 10 mg/kg ESC, or 10 mg/kg BUP in a drug-paired context (CS+) on alternating days (10 days total, 5 pairings in each context). One week later, rats were exposed to 3 sessions of forced swim stress (FSS) over 3 consecutive days (FSS_{1-3}; 10 mins/session), with exposure to the CS- and CS+ prior to FSS_2 and FSS_3, respectively, and immobility was recorded on each session. In Experiment 2, the same conditioning protocol was employed to conducted to assess unbiased conditioned place preference and investigate conditioned locomotion and conditioned approach to a KET-paired context. It was found that exposure to the CS+ paired with KET, but not ESC, or BUP reduced immobility without affecting horizontal motor activity. Moreover, the KET CS+ did not produce conditioned approach (or withdrawal), indicating that the anti-immobility effect of the KET CS+ context was not the byproduct of conditioned hyperactivity. Therefore, the current data suggests that KET, differently from other common antidepressant medications, can elicit a conditioned antidepressant response which may play a role in its sustained therapeutic effects.

Disclosures: B. Melanson: None. **M. Wolter:** None. **Z. Leatham:** None. **T.D. Lapointe:** None. **F. Leri:** None.

Poster

P734. Learning and Memory in Addiction II

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Program #/Poster #: P734.05

Topic: G.09. Drugs of Abuse and Addiction

Support: Natural Sciences and Engineering Research Council of Canada

Title: Inhibition of noradrenergic and corticotrophin-releasing factor systems block enhancement of memory consolidation by heroin withdrawal and conditioned heroin withdrawal

Authors: *N. BAIDOO[1], F. LERI[2];
[1]Univ. of Guelph, Guelph, ON, Canada; [2]Psychology, Univ. Guelph, Guelph, ON, Canada

Abstract: AIM: It is well established that opioid withdrawal can be associated to environmental stimuli through classical conditioning to produce conditioned withdrawal. We have recently found that conditioned withdrawal has significant cognitive effects as, similarly to acute precipitated withdrawal, it enhances consolidation of memory. The aim of the current study was to explore the role of noradrenaline and corticotrophin-releasing factor (CRF) in memory modulation induced by unconditioned and conditioned heroin withdrawal. **METHODS:** Male Sprague-Dawley rats were implanted with osmotic mini-pumps releasing 3.5 mg/kg/day heroin, and received injections of 3 mg/kg naloxone preceded by 0.1 - 0.6 mg/kg lofexidine (alpha-2 adrenergic agonist) or 10 - 20 mg/kg antalarmin (CRF 1 receptor antagonist) immediately after the sample phase of spontaneous object recognition memory task. To produce conditioned withdrawal, a contextual conditioning procedure was employed whereby heroin-exposed rats were confined for 2 hours in a context (CS+) following injections of 3 mg/kg naloxone and in another context (CS-) following vehicle injections. Seven days after removal mini-pumps, the effects of immediate exposure to the CS+ (or CS-) preceded by 0.6 mg/kg lofexidine or 20 mg/kg antalarmin were tested. **RESULTS:** It was found that both lofexidine and antalarmin blocked the enhancement of object memory consolidation by post-training naloxone precipitated withdrawal and by conditioned withdrawal. **CONCLUSIONS:** These experiments suggest that pharmacological and psychological withdrawal have significant effects on memory storage by activating noradrenergic and CRF systems.

Disclosures: N. Baidoo: None. **F. Leri:** None.

Poster

P734. Learning and Memory in Addiction II

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Program #/Poster #: P734.06

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant 5R01DA047368-03

Title: The Effects of Escalated Fentanyl Intake on Decision-Making Dynamics

Authors: *T. K. SHAVER, M. J. STEPHENS, M. A. HALLORAN, J. BECKMANN;
Psychology, Univ. of Kentucky, Lexington, KY

Abstract: Opioid Use Disorder (OUD) is a major public health problem in the United States. There is evidence to suggest that substance use disorders are, at least partially, disorders of decision-making, consisting of persistent drug choice over alternatives. As such, two competing

hypotheses regarding the role of decision-making in OUD are: 1) OUD is representative of a deficit in decision-making processes; 2) OUD is the result of relative value processes. In order to tease apart hypotheses above, we utilized both isomorphic (food-food) and allomorphic (remifentanil-food) choice in tandem with 1-hr and 6-hr fentanyl access, a preclinical model of escalated opioid intake. If OUD is the result of decision-making deficits, 6-hr fentanyl access should disrupt isomorphic decision-making. Conversely, if OUD is representative of relative value processes, 6-hr fentanyl access should systematically shift allomorphic choice, while leaving isomorphic choice unaffected. First, rats were trained on either a food-food (isomorphic) or remifentanil-food (allomorphic) choice procedure, where both the magnitude (1, 2, and 4 food pellets or 1, 3, and 10 ug/kg of remifentanil) and probability of outcomes were manipulated unpredictably, until choice was stable. Rats were then trained on a fentanyl self-administration (0.25 ug/kg to the animal's weight) under an FR1 schedule with 1-hr access until intake was stable. Rats were then split into 1-hr and 6-hr fentanyl access groups, matched for both choice performance and fentanyl acquisition intake; each group was subsequently trained such that they underwent choice (isomorphic or allomorphic) in the morning and fentanyl access (1-hr or 6-hr) one hour after completing choice. The results to date suggest that 6-hr fentanyl access had no effect on isomorphic choice; however, 6-hr fentanyl access systematically shifted allomorphic choice, producing increased sensitivity to changes in remifentanil dose during choice. Collectively, the current results suggest that OUD may be the result of relative valuation processes, not necessarily representative of a deficit in decision-making processes generally.

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Poster

P746. Cognitive and Behavioral Effects of Cannabinoids

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Program #/Poster #: P746.01

Topic: G.09. Drugs of Abuse and Addiction

Support: Constantine Douketis Award

Title: Acute Stress Effects on Mood, Cannabis Urges and Motives in Heavy Cannabis Users

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Abstract: Many people report using cannabis as a way to cope with stress. Acute, low doses of THC have been reported to induce stress relieving effects, which may drive continued use for

individuals looking to cope with stress. It is unclear whether heavy cannabis users have alterations in their stress response, and whether this relates to coping motivations. Currently, there is also limited data on how stress reactivity may play a role in cannabis craving. Therefore, the aim of the current study is to assess the effects of acute stress on mood and cannabis craving in individuals who self-report heavy cannabis use. Participants included 56 heavy cannabis users and 50 healthy controls who were randomly assigned to stress and no-stress conditions. Participants completed mood questionnaires, from which a total mood disturbance score was calculated, and questionnaires on cannabis use urges throughout the experiment. Cannabis users also completed a questionnaire on motives for cannabis use. Participants assigned to the stress condition underwent a validated psychosocial stressor. Acute stress increased self-report total mood disturbance scores in both control and cannabis groups, however, the cannabis group consistently reported higher total mood disturbance, including at baseline and throughout the experimental session. Acute stress had no effects on cannabis craving; however, craving was greater in cannabis users in the no-stress conditions, compared to cannabis users in the stress condition. Within the stress condition, higher coping motives for cannabis were consistently correlated with greater total mood disturbance scores across the experimental session. Our findings shed light on momentary subjective stress responses in cannabis users and reinforce trait-like patterns of stress reactivity. These results reinforce previous findings of elevated stress in heavy cannabis users, and demonstrate links with coping motives. Further, these data show that acute stress does not induce cannabis craving; unexpectedly, craving scores were elevated in the no-stress cannabis group without the presence of a stressor. These findings suggest that craving may be a result of trait-like, rather than state factors. Future studies can examine the underlying physiological mechanisms, including relationships with specific cannabinoids as well as the time course of reactivity alterations in heavy cannabis users.

Disclosures: S.L. Brassard: None. **K. Manuja:** None. **J. MacKillop:** None. **I.M. Balodis:** None.

Poster

P746. Cognitive and Behavioral Effects of Cannabinoids

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P746.02

Topic: G.09. Drugs of Abuse and Addiction

Support: Colorado Department of Public Health and Environment 96947

Title: Acute effects of legal market high potency THC-dominant vs. CBD-dominant cannabis concentrates: objective and subjective intoxication outcomes

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Abstract: As the market for cannabis concentrate products continues to grow, the lack of research regarding the combined and differential effects of concentrated forms of the two most prominent cannabinoids, THC and CBD, becomes more glaring. The present study analyzes cannabinoid blood levels and subjective outcomes of physical sensation and affective state after *ad libitum* use of legal market concentrate products. Participants were randomly assigned to either a THC or CBD-dominant concentrate product and completed a baseline session, followed by an experimental mobile laboratory session consisting of 3 timepoints - before, immediately after, and one-hour after *ad libitum* concentrate use. As expected, the THC-dominant concentrate induced higher intoxication effects, as well as higher ratings of drug effect and drug reward than the CBD-dominant concentrate. Both products induced immediate feelings of elation that diminished over the subsequent hour. Differential subjective outcomes were seen between products, with the CBD-dominant group seeing immediate decreases in tension and anxiety, while the THC-dominant group saw significant decreases in anxiety only after one hour. Paranoia spiked immediately post-use in the THC-dominant concentrate group, which decreased back to baseline levels with an hour. Overall, the positive effects of the CBD-dominant concentrate on mood accompanied a lower level of intoxication and absence of undesirable effects such as paranoia experienced with the THC-dominant concentrate. The results herein support the need for further investigation of the harm-reduction potential of concentrated CBD, both alone and in conjunction with THC.

Disclosures: M. Drennan: None. **H. Karoly:** None. **L. Bidwell:** None.

Poster

P746. Cognitive and Behavioral Effects of Cannabinoids

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P746.03

Topic: G.09. Drugs of Abuse and Addiction

Title: Transcranial Magnetic Stimulation as an intervention for cannabis use disorder in undergraduates

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Abstract: Cannabis use is globally prevalent among college undergraduates. According to a 2016 survey, 35.3% of young adults between 19 and 28 years of age use marijuana at least annually (Schulenberg et al., 2016). Importantly, nearly 25% of U.S college students meet the requirements for a clinical diagnosis of cannabis use disorder (CUD) (Caldeira et al., 2008). CUD is associated with adverse consequences such as impaired cognition, poor academic performance, lower income, and lower IQ (Pearson, et al., 2017). Additionally, the misuse of cannabis is correlated with increased anxiety and depression (Volkow et al., 2014). With the legalization of cannabis spreading across the U.S, it is imperative that informed research be conducted regarding the treatment of CUDs in young adults.
TMS is a noninvasive method of neural modulation that when used to stimulate the dorsolateral prefrontal cortex (DLPFC), has been shown to reduce nicotine cravings (Hauer et al., 2019). The theory behind its effectiveness in reducing substance cravings involves the stimulation of dopaminergic reward pathways (Sahlem et al., 2018). The purpose of the current study is to investigate the effects of TMS to DLPFC on cannabis cravings and use in college undergraduates who are at risk for CUD.
Sixty undergraduates who meet clinical diagnosis for CUD and want treatment are being recruited for two in-person TMS sessions and an online follow-up one week later. We are using a within-subjects design where the participant receives either active or sham TMS for the first session, and then receives the opposite condition for the second session. Prior to receiving either stimulation condition, participants complete the marijuana craving questionnaire (MCQ). The active TMS session stimulates left DLPFC with intermittent Theta Burst Stimulation (iTBS) for 3 minutes at 100% of motor threshold. Before, during, and after stimulation, participants look at various cannabis stimuli and complete the MCQ again at the end of the session. So far, we have collected data on 4 participants. The stimulation is well-tolerated, and we see reductions in marijuana cravings in 75% of participants. Data collection is ongoing, but we hypothesize that participants will experience reduced cannabis cravings after the iTBS at the end of the session and reduced real life cannabis use compared to the sham stimulation condition. Our aim is to produce an effective and noninvasive intervention to help reduce CUDs in college students.

Disclosures: J.M. Kelly: None. **R.S. Astur:** None. **S.M. Sklenarik:** None. **F. Hoeft:** None. **V.R. Steele:** None.

Poster

P746. Cognitive and Behavioral Effects of Cannabinoids

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Program #/Poster #: P746.04

Topic: G.09. Drugs of Abuse and Addiction

Title: The neurological effects of recent cannabis use on processing emotional facial expressions: An ERP study

Authors: H. DAMRA, B. DIXON, *R. TORRENCE;
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Abstract: Cannabis use has residual effects on neurological responses to fearful facial expressions. Other research has shown that cannabis can have acute effects on cognition for 24 hours after use. This study examined the effects of cannabis use within the last 24 hours on processing fearful faces as measured by the event-related potential, N170. We had 16 participants that were identified as cannabis users and 9 of these participants used cannabis with 24 hours (but not with 8 hours) of the study. The participants were asked to view emotional facial expressions and identify the emotion (either fear, neutral, or happy). We used NeuroScan to acquire the ERP data. An average reference was used electrode P8 with a time window of 150-190 ms for N170. Three separate t-tests were used (Bonferroni correction was used to adjust critical p-value, $p = .016$) to examine group differences within each condition. The N170 amplitude for the participants without recent cannabis use was significantly more enhanced than the participants with recent use for fear ($t(14) = 2.955$, $p = .010$) and happy ($t(14)$ 3.225, $p = .006$), but not neutral ($t(14) = 2.529$, $p = .024$). The results indicate that cannabis use within 24 hours reduces face processing.

Disclosures: H. Damra: None. **B. Dixon:** None. **R. Torrence:** None.

Poster

P746. Cognitive and Behavioral Effects of Cannabinoids

Location: Virtual Only

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Program #/Poster #: P746.05

Topic: G.09. Drugs of Abuse and Addiction

Support: UW Madison Analytical Instrumentation Center

Title: Development of Cross-Reactive Antibodies for the Identification and Treatment of Synthetic Cannabinoid Receptor Agonist Toxicity

Authors: *A. WOROB, C. WENTHUR;
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Abstract: Synthetic cannabinoid receptor agonists (SCRAs) are compounds which mimic the pharmacology of psychoactive components in cannabis. These compounds are inexpensive, commercially available and difficult to identify with modern analytical methods, making them highly accessible for recreational use. The diverse nature of SCRA structures contributes to their unique pharmacological profile, resulting in increased potency and toxicity compared to cannabis. Suspected SCRA toxicity, which can present with a breadth of cardiovascular, gastrointestinal and neurological disturbances, is currently addressed through symptom

management followed by toxicological screening after patient discharge. We can address the dual need for improved diagnostic and therapeutic intervention against SCRA toxicity with antibodies generated in the presence of SCRA-resembling small molecule haptens conjugated to immune-activating proteins. Similar antibody strategies employed for opioids, nicotine and other commonly misused drugs have shown potential in both detecting these drugs *in vitro* and sequestering them to the periphery *in vivo*, blunting their psychotropic effects. As most SCRAs incorporate one or more previously identified structural motifs across their tail, core, linker and head group, we generated antibodies in mice against haptens incorporating several common motifs that have been observed across different generations of SCRAs. Vaccinated animals were then challenged with structurally matched SCRAs to determine if the vaccines could prevent SCRA-induced physiological changes in locomotion, body temperature and nociception. Furthermore, these antibodies were then tested against a panel of SCRAs with a broader range of structural congruency to the test haptens, using competitive ELISA methods, to evaluate their diagnostic potential. We determined that haptens containing valine-like head groups linked to a heterocyclic core generated robust immune responses resulting in antibodies capable of binding SCRAs with bioisosteric changes to the core and tail groups compared to the hapten. Contrastingly, our naphthyl head group haptens stimulated considerably weaker immune responses, resulting in antibodies with poor potency and little ability to bind even structurally similar SCRAs. Future expansion of hapten diversity to address cross-reactivity of antibodies within and across structural families is thus warranted, and such efforts are underway.

Disclosures: A. Worob: None. **C. Wenthur:** None.

Poster

P746. Cognitive and Behavioral Effects of Cannabinoids

Location: Virtual Only

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Program #/Poster #: P746.06

Topic: G.09. Drugs of Abuse and Addiction

Support: R15MH122729-01

Title: Investigating the effect of cannabidiol on habit-formation and striatal dopamine release

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Abstract: Habits are inflexible behaviors that persist despite changes in action outcomes or outcome value. While habits allow for efficient behavioral responding, neuropsychiatric diseases such as drug addiction and obsessive-compulsive disorder are characterized by overreliance on habits and overactive brain regions involved in habit formation. The transition from goal-

directed to habitual responding is mediated by the striatum and dopamine inputs to the striatum. Additionally, the CB1 cannabinoid receptor is necessary for both habit formation and normal dopamine release. The commercially popular drug cannabidiol (CBD) may influence habit formation by decreasing CB1 receptor efficiency, by modifying striatal dopamine release, or by a serotonin-mediated mechanism. To explore this, we trained mice on random ratio (RR20) or random interval (RI60) schedules designed to elicit goal-directed or habitual lever-pressing, respectively. Throughout training and habit probes, mice were administered either CBD (20 mg/kg) or vehicle as a control. Mice were tested for habitual responding using probe trials following reinforcer-specific devaluation as well as omission trials, where mice had to withhold responding to earn rewards. Our results suggest that CBD modestly increased goal-directed responding in the random interval but not the random ratio group. Further, we utilized the optical dopamine sensor dLight to conduct fiber photometry recordings of real-time changes in dorsal striatal dopamine levels in an open field exploratory task before and after 20 mg/kg CBD injections. CBD did not significantly modify the amplitude or frequency of spontaneous dopamine events, suggesting alterations in habit formation may be due to a dopamine-independent mechanism. These results indicate CBD may be a potential therapeutic agent in disorders characterized by maladaptive habit formation and that CBD does not modify habits through changes in striatal dopamine levels.

Disclosures: C.R. Morehouse: None. **C.J. Maddox:** None. **R. McLaughlin:** None. **J.R. Scott:** None. **E.S. Ramsson:** None. **C.D. Howard:** None.

Poster

P746. Cognitive and Behavioral Effects of Cannabinoids

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Program #/Poster #: P746.07

Topic: G.09. Drugs of Abuse and Addiction

Support: Funding provided by Ellipse Analytics

Title: Effects of oral cannabidiol administration on sleep and pain responses and potential underlying pathways in male rats

Authors: *J. M. SKULLY[1], J. KINA[2], A. TROMBLEY[2], A. PALOPOLI[2], L. M. RICHARDSON[2], N. KULAGLIC[2], C. M. WALLIN[1], S. BRUMMELTE[1], S. BOWEN[1]; [1]psychology, [2]Wayne State Univ., Detroit, MI

Abstract: There has been an increase in popularity for Cannabidiol (CBD), the non-psychoactive component of Marijuana. Research assessing CBD's use as a therapeutic (e.g., anxiolytic, analgesic, anti-inflammatory, anti-stress, etc.) in both human and animal models showed some promising results, however, CBD's effects and mechanisms of action still need

further exploration. This project aimed to evaluate the potential therapeutic outcomes associated with administration of a hemp derived 99% pure CBD compound provided from Ellipse Analytics (Denver, CO). Moreover, we sought to evaluate CBD's influence on brain-derived neurotrophic factor (BDNF) and transient receptor potential cation channel subfamily V member 1 (TRPV1) expression. CBD (0, 20, or 40 mg/kg) was administered by oral gavage to male rats over 10 days (n=16/group), with behavioral assays being run on the 1st, 5th, and 10th day of administration to assess pain and sleep behaviors. Rats were sacrificed on day 11, and brains were collected for analysis. Western blotting will be used to analyze protein concentrations (pro-BDNF, BDNF, TRPV1) in different brain regions of interest. Behavioral results revealed an effect of the 20mg/kg, but not 40mg/kg dose of CBD to reduce the time spent in an inactive sleep state and to increase response time to a heat stimulus. These findings offer further support for a potential therapeutic effect of CBD on sleep regulation and anti-nociception and future brain analysis will provide insight on the potential involvement of the BDNF and TRPV1 pathways in producing CBD's effects. Further studies are necessary to assess dose-dependent effects with acute or prolonged exposures, as well as study the effectiveness in females.

Disclosures: J.M. Skully: None. **J. Kina:** None. **A. Trombley:** None. **A. Palopoli:** None. **L.M. Richardson:** None. **N. Kulaglic:** None. **C.M. Wallin:** 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.; Ellipse Analytics. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Ellipse Analytics. **S. Brummelte:** None. **S. Bowen:** 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.; Ellipse Analytics. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Ellipse Analytics.

Poster

P746. Cognitive and Behavioral Effects of Cannabinoids

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Topic: G.09. Drugs of Abuse and Addiction

Support: DGAPA UNAM IN232120, CONACyT (1082544)

Title: Effects of CB2 receptor activation in the anterior cingulate cortex on binge-type behavior induced by limited acces to a palatable diet.

Authors: *J. RODRÍGUEZ-AGUILAR[1], L. RODRIGUEZ-SERRANO[2], F. CORTÉS SALAZAR[3], J. MANCILLA-DIAZ[4], R. ESCARTIN-PEREZ[1];
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Abstract: The anterior cingulate cortex (ACC) plays a role in processing the relationship between motivational and emotional information. Moreover, the ACC has connections to the nucleus accumbens (NAc) and ventral tegmental area, structures of the mesocorticolimbic system that participate in the modulation of mechanisms controlling reward. The endocannabinoid system contributes differentially to the modulation of reward and aversion, depending on the receptor activated. Activation of CB1 receptors (CB1r) induces rewarding effects, while activation of CB2 receptors (CB2r) induces aversive effects. Consistently, some studies reported that the activation of the CB1r increases food intake, while CB2r activation has an inhibitory effect on feeding. Furthermore, rats with binge-type behavior induced by restricted access to a palatable diet have altered the tone of particular endocannabinoids (2-arachidonoylglycerol and Anandamide) in specific brain areas related to the perception of the hedonic properties of palatable foods (prefrontal cortex,NAc, amygdala). Considering that ACC expresses CB2r, we hypothesized that activation of CB2r in the ACC would decrease consumption of a palatable diet. Accordingly, this study aimed to evaluate the effects of CB2r activation in the ACC on the consumption of a palatable diet in male Wistar rats. The standard diet (SD, chow) was available ad libitum, while the palatable diet (PD, evaporated milk added with 10% sucrose) was provided 60 min per day, during 35 days in the light phase of the light/dark cycle. We measured thedaily SD consumption (24 h) and the SD intake during the 60 min previous to the presentation of the PD. Then, we measured the consumption of PD (60 min). On day 20, when the consumption of the PD stabilized (variations less than 20% in 3 consecutive sessions), microinjection cannulas were stereotaxically implanted in the ACC, and animals continued accessing the PD for eight additional days after recovery (n= 8). Subsequently, rats received intra-ACC injections of the CB2 receptor agonist (GW838972A, 0.25 µg/µl),the CB2 receptor antagonist (AM630, 0.75 µg/µl) or both (GW838972A, 0.25 µg/µl+ AM630, 0.75 µg/µl) 15 min before the access to the PD and food intake was measured (SD and PD, 60 min). We found that rats with limited access to the PD (60 min per day) increased their energy intake (>50% from the total daily caloric intake), expressing binge-like eating after 31 days of limited access to the PD. Unexpectedly, we found that activation of CB2r in the ACC with GW838972A did not change the PD intake. We conclude that the activation of CB2r in the ACC does not prevent the induced binge-type eating.

Disclosures: J. Rodríguez-Aguilar: None. **L. Rodriguez-Serrano:** None. **F. Cortés Salazar:** None. **J. Mancilla-Diaz:** None. **R. Escartin-Perez:** None.

Poster

P746. Cognitive and Behavioral Effects of Cannabinoids

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH grant 1P01DA041307-01
NIH grant R01CA142115-02

Title: Strain comparisons to activation of CB2 receptors as a prevention for morphine induced respiratory depression

Authors: *B. WIESE[1,2], A. ALVAREZ REYES[1], S. NIKAS[2], L. JI[2], Y. LIU[2], A. MAKRIYANNIS[2], T. VANDERAH[1], T. LARGENT-MILNES[1];
[1]Univ. of Arizona, Tucson, AZ; [2]Northeastern Univ., Boston, MA

Abstract: Strain comparisons to activation of CB2 receptors as a prevention for morphine induced respiratory depression
Beth M. Wiese[1], Angelica Alvarez Reyes[1,2], Spyros P. Nikas[3], Lipin Ji[3], Yingpeng Liu[3], Alexandros Makriyannis[3], Todd W. Vanderah[1], Tally M. Largent-Milnes[1]
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Introduction: With recent publications of a CB2 agonist preventing opioid induced respiratory depression (OIRD) in CD1 male mice, there is urgency to determine if this is preserved across strains to continue movement to reduce opioid harms in humans. **Objective:** Cannabinoid receptor-2 (CB2R) agonism is reported to prevent OIRD in CD1 mice, yet whether this holds true across strains in the C57 mouse is unknown.**Methods:** Utilizing whole-body plethysmography chambers, sought to evaluate the influence of CB2 activation on respiratory depression using selective agonists alone and in combination with morphine across C57 wild-type and knock-out mice, compared to CD1 male mice. **Results:** AM2301, the novel CB2 agonist did not induce respiratory depression across strains. Moreover, it significantly attenuated morphine -induced respiratory depression in C57 mice, as it did in CD1 mice. **Conclusion:** Building on past literature, findings suggest prevention of OIRD via CB2R activation is maintained across strains, supporting CB2R agonism as a possible harm reduction method in opioid therapy.

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Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH P20GM113109

Title: Effects of Edible Delta-9-Tetrahydrocannabinol at Low Doses on Locomotor Behavior in Adult Rats

Authors: *M. C. AZUMA[1], D. A. LAUX[2], T. J. MOSER[3], M. CAIN[4];
[2]Psychological Sci., [3]Dept. of Psychological Sci., [1]Kansas State Univ., Manhattan, KS;
[4]Psychological Sci., Kansas Sate Univ., Manhattan, KS

Abstract: Recent state legalization and acceptance of cannabis across the US has made it one of the most commonly used illicit drugs. Using edible forms of cannabis has become an increasingly popular method of consumption. Previous literature has primarily investigated behavioral changes resulting from inhalation, intraperitoneal, subcutaneous, and intragastric administration of delta-9-tetrahydrocannabinol (THC), the main psychoactive component of cannabis. Each has been tested using similar dose ranges (5 - 20 mg/kg). However, medical use of cannabis in edible form is commonly prescribed at doses of 0.1 - 1 mg/kg. Importantly, THC is metabolized differently when ingested compared to the other methods of use. Therefore, it is imperative to investigate the behavioral and neurobiological changes following ingestion of low doses of THC. In the current experiment, we utilized a rodent model of edible THC across a range of low doses to determine if it would alter locomotor activity in an open-field arena (OFA). Male Sprague Dawley adult rats received vehicle- or THC- dissolved cookies (0.625, 1.25, and 2.5 mg/kg) 30min prior to each 2-hour OFA test. Eight sessions were performed every other day for a total of 16 days. On rest days, all animals consumed vehicle- dissolved cookies. Our data suggest that there is a dose-dependent increase in distance traveled (cm) following ingestion of low doses of THC during the initial sessions. Interestingly, by the final session, vehicle and all doses of THC result in similar patterns of locomotor activity, suggesting behavioral desensitization to low dose THC. Previous data has shown that high THC doses in edible form dose dependently decrease locomotor activity. The highest dose tested in the current experiment is comparable to the lowest doses used in past research with edibles along with other administration routes. The results of our current experiment suggest dose-dependent locomotor effects from using THC at high vs low doses in edible form.

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Poster

P752. Cocaine: Cellular and Neurophysiological Mechanisms

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Program #/Poster #: P752.01

Topic: G.09. Drugs of Abuse and Addiction

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NIH Grant DA044308
NIH Grant DA051551
NARSAD Young Investigator Grant

Title: Depletion of the gut microbiomealters transcriptional homeostasis and reduces persistence of cocaine sensitization

Authors: *K. R. MECKEL[1], R. S. HOFFORD[2], N. L. MERVOSH[2], N. TUKURU[2], J. B. HANKS[3], J. J. FAITH[3], P. ROUSSOS[3], D. D. KIRALY[4];
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Abstract: Psychostimulant addiction represents a devastating public health crisis leading to extensive morbidity and mortality. Despite this, there are currently no FDA-approved medications for treatment of psychostimulant use disorders. A growing body of literature suggests that the bacterial composition of the gut microbiome significantly affects brain and behavior in animal models of psychiatric disease. Prior work from our laboratory has demonstrated that depletion of the gut microbiome significantly affects the formation of cocaine conditioned place preference and locomotor sensitization. However, no currently published studies have examined the role of the gut microbiome in modulating the persistence of cocaine-induced changes in neuronal or behavioral plasticity. Given that the prevention of relapse following abstinence is the most clinically challenging issue in treating patients with substance use disorders, studies examining the effects of microbiome manipulations in relapse-relevant animal models are critical for the development of translational strategies in this space. For these studies mice had their microbiome depleted with non-absorbable antibiotics in their drinking water and were compared to untreated controls. Locomotor sensitization was induced with five daily injections of cocaine, and after 28 days of withdrawal a cocaine challenge was given to assess the persistence of locomotor sensitization. Despite normal development of sensitization, antibiotic-treated mice exhibited significantly reduced persistence of locomotor sensitization after prolonged withdrawal. To identify molecular signatures underlying these behavioral changes, animals were sacrificed one hour after a challenge injection of cocaine, and the nucleus accumbens was dissected out for RNA-sequencing analysis. While cocaine-challenged control animals showed changes in approximately 200 genes, antibiotic-treated mice had significant changes in 3,610 genes. To further interrogate the effects of microbiome composition on behavior, locomotor sensitization to cocaine was induced in germ-free mice and compared to specific-pathogen-free controls. Animals were maintained under gnotobiotic conditions for the duration of the study, and germ-free mice demonstrated altered persistence of sensitization after 28 days of withdrawal compared to controls with an intact microbiome. Taken together, these findings suggest that the microbiome may serve as a key homeostatic regulator of behavioral

response and gene expression in the brain, and suggest that the microbiome has potential as a translational research target.

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Poster

P752. Cocaine: Cellular and Neurophysiological Mechanisms

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Topic: G.09. Drugs of Abuse and Addiction

Support: R01NS106056-03

Title: Changes in DNA methylation underlie differences in extinction of cocaine conditioned place preference

Authors: *M. R. BAKER, C. E. BURGDORF, F. TAKI, E. BRINDLEY, A. RAJADHYAKSHA, M. TOTH;
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Abstract: Cocaine addiction is a chronic condition in which craving and subsequent drug seeking can be elicited by drug-associated cues. In rodents, this can be modeled using conditioned place preference (CPP), where animals acquire a preference for a chamber where they receive cocaine. Once place preference is acquired, the drug-associated memory can be extinguished by repeatedly exposing animals to the previously cocaine-paired environment in the absence of the drug. However, some mice fail to extinguish, continuing to prefer the previously cocaine-paired side even without the drug. Animals that fail to extinguish the previously drug-associated memory could provide insight to individual differences in vulnerability to drug dependence. One possible underlying mechanism is DNA methylation. To first identify the epigenetic differences between these groups, we analyzed the methylomes of the dorsal dentate gyrus of Extinction and Failed Extinction male mice. These animals showed differentially methylated regions (DMRs; genomic regions with significantly different methylation between groups). Genome-wide methylation analysis showed that acquiring cocaine CPP led to a shift from bimodal methylation (i.e. either unmethylated (0% methylation) or methylated (100% methylation)) to intermediate levels of methylation (methylation between 15-85%) in the animals that acquired cocaine CPP. This intermediate level then shifted to hyper- or hypomethylated after successfully extinguishing the drug-paired memory. An Ingenuity Pathway Analysis of extinction DMRs showed enrichment for genes within an activity-dependent calcium and glutamate pathway. One differentially methylated gene in this pathway is *cacna1c*, which encodes the $Ca_v1.2$ isoform of the L-type voltage-gated calcium channel, a gene implicated in

drug dependence. One DMR in this gene had decreased methylation in the mice that extinguished compared to those that did not. This DMR maps onto a putative distal enhancer, so we tested the effect of this region and its methylation on the activity of a reporter gene. We found that, as predicted, this DMR demonstrated enhancer activity in vitro that was decreased by methylation. Supporting this methylation data, there was an increase in *cacna1c* mRNA and protein in the animals that successfully extinguished that was not present in the animals that failed to extinguish. Taken together, this preliminary work suggests differences in DNA methylation within the dorsal dentate gyrus could dictate underlying differences that lead to a propensity to develop drug dependence.

Disclosures: M.R. Baker: None. **C.E. Burgdorf:** None. **F. Taki:** None. **E. Brindley:** None. **A. Rajadhyaksha:** None. **M. Toth:** None.

Poster

P752. Cocaine: Cellular and Neurophysiological Mechanisms

Location: Virtual Only

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Program #/Poster #: P752.03

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01DA040965
NIH Grant R21DA045277

Title: Cocaine memory reactivation induces functional adaptations within parvalbumin interneurons in the rat medial prefrontal cortex

Authors: *E. T. JORGENSEN[1], A. E. GONZALEZ[3], J. H. HARKNESS[5], D. M. HEGARTY[6], A. THAKAR[2], J. AADLAND[1], S. A. AICHER[7], B. A. SORG[4], T. E. BROWN[2];
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Abstract: Substance use disorder is a complex disease created in part by maladaptive learning and memory mechanisms following repeated drug use. Exposure to drug-associated stimuli engages prefrontal cortex circuits; and dysfunction of the medial prefrontal cortex (mPFC) is thought to underlie drug-seeking behaviors. Growing evidence supports a role for parvalbumin containing fast-spiking interneurons (FSI) in modulating prefrontal cortical microcircuit activity by influencing the balance of excitation and inhibition, which can influence learning and memory processes. Most parvalbumin FSIs within layer V of the prelimbic mPFC are surrounded by specialized extracellular matrix structures called perineuronal nets (PNN). Previous work by our group found that cocaine exposure altered PNN-surrounded FSI function,

and pharmacological removal of PNNs reduced cocaine-seeking behavior. However, the role of FSIs and associated constituents (parvalbumin and PNNs) in cocaine-related memories was not previously explored and is still unknown. Here, we found that reactivation of a cocaine conditioned place preference memory produced changes in cortical PNN-surrounded parvalbumin FSIs, including decreased parvalbumin intensity, increased parvalbumin cell axis diameter, decreased intrinsic excitability, and increased excitatory synaptic input. Further investigation of intrinsic properties revealed changes in the inter-spike interval, membrane capacitance, and afterhyperpolarization recovery time. Changes in these specific properties suggest an increase in potassium-mediated currents, which was validated with additional electrophysiological analysis. Collectively, our results indicate that cocaine memory reactivation induces functional adaptations in PNN-surrounded parvalbumin neurons, which likely alters cortical output to promote cocaine-seeking behavior.

Disclosures: E.T. Jorgensen: None. **A.E. Gonzalez:** None. **J.H. Harkness:** None. **D.M. Hegarty:** None. **A. Thakar:** None. **J. Aadland:** None. **S.A. Aicher:** None. **B.A. Sorg:** None. **T.E. Brown:** None.

Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: R01DA041513

Title: Differential effects of short and extended cocaine access on astrocytes in rat nucleus accumbens

Authors: *M.-F. XIA[1], P. I. ORTINSKI[2];
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Abstract: Nucleus accumbens is involved in motivational and affective behavioral processing, and plays a significant role in the development and maintenance of substance use. The release of dopamine from the ventral tegmental area into the nucleus accumbens regulates incentive salience and facilitates reinforcement and reward-related motor function learning. Recent studies reveal that, in the context of cocaine exposure, dopamine transmission in the ventromedial striatum is pivotal in the control of initial drug use. In addition, astrocyte morphology has been shown to be affected by cocaine, but the functional role of astrocytic signaling in cocaine use remains unknown. The goal of this study is to address astrocytic adaptions within nucleus accumbens under two different cocaine exposure regimes.We have observed morphological and functional changes under elevated extracellular dopamine in cultured astrocytes. Physiological

increases in dopaminergic signaling over the course of cocaine self-administration were shown to alter astrocyte morphology and expression profile of genes.To characterize the astrocytic effects on cocaine-induced dopamine elevation and how it contributes to neural circuit functions, we used a rat self-administration model. Two groups of animals went through short- (1h/day) and extended- (6h/day) access cocaine self-administration for 14 days. To identify the functional effects of cocaine on astrocytes, we imaged astrocytes expressing calcium indicator, GCamp6f and performed whole-cell patch-clamp recordings.We noted the extended-access to cocaine leads to an elevation of drug-taking behavior, but not in the short-access group. The intracellular level of calcium within NAc astrocytes is differentially affected by short- or extended- access cocaine self-administration. Extended exposure significantly decreased the amplitude of spontaneous Ca2+ transients at both plasma membrane and endoplasmic reticulum. Calcium transients at two compartments were differently affected by mGluR or IP3 antagonists, suggested compartmentalized mechanisms of calcium signaling regulation. On-going experiments examine how astrocyte membrane properties are regulated by cocaine exposure regimes and how cocaine may alter coupling within astrocytic networks.Astrocytes adapt to cocaine exposure differently through short- and extended- access self-administration, both morphologically and functionally. Our results indicate a potential regulatory role of astrocytic signaling in the NAc on neural mechanisms underlying substance use.

Disclosures: M. Xia: None. **P.I. Ortinski:** None.

Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant F31 DA050422
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Title: The effects of cocaine self-administration on astrocyte mitochondria morphology

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Abstract: Rat cocaine self-administration leads to both structural and functional impairments in astrocytes in the nucleus accumbens (NAc), a region key to the brain's reward circuitry. In particular, cocaine impairs the ability of NAc astrocytes to maintain glutamate homeostasis, the balance between extra-synaptic and synaptic levels of glutamate. This impairment is mediated in

part by decreased expression of the astroglial glutamate transporter GLT-1, which is responsible for 90% of synaptic glutamate uptake. Glutamate uptake carries a high energetic cost, which is met by mitochondria. Mitochondria colocalize with GLT-1 in astrocytes and inhibition of mitochondria in astrocyte cultures reduces glutamate uptake. Moreover, degradation of astrocytic processes has been attributed to reduced mitochondrial function in cultured astrocytes. Given the cocaine-induced impairments in glutamate homeostasis and altered astrocyte morphology, and the role mitochondria may play in both of these processes, it is reasonable to hypothesize that astrocytic mitochondria function may be compromised following cocaine self-administration. To investigate this hypothesis, confocal imaging of NAc astrocytes was performed using two AAV5s: mitochondrially-targeted enhanced green fluorescent protein as well as a membrane-targeted Td-tomato fluorescent protein, both under control of an astrocyte-specific promoter in male rats, 24hrs following 12 days of short-access (2hrs/day) cocaine self-administration. Morphological properties of mitochondria were assessed using the MitochondriaAnalyzer ImageJ plugin. Results indicate that following cocaine self-administration mitochondria in NAc astrocytes exhibit significantly increased volume, surface area, and diameter as compared those within saline self-administering animals. This is likely indicative of oxidative stress; however, further analysis is needed. Interestingly, this effect was not observed in a separate cohort of rats trained in 12 days of self-administration and 16-17 days of extinction, suggesting this effect is associated with early abstinence, similarly as has been reported for dendritic mitochondria. Experiments designed to test the effect of sex on this observation, as well as expression of mitochondria stress-related genes and markers of oxidative stress in astrocytes, are ongoing.

Disclosures: E.A. Witt: None. **A. Testen:** None. **J.G. Jackson:** None. **K.J. Reissner:** None.

Poster

P752. Cocaine: Cellular and Neurophysiological Mechanisms

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA023206
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Title: Astrocyte proximity to glutamate synapses in the rat nucleus accumbens core: effect of cocaine self-administration

Authors: *S. SESACK[1], T. PLUTE[1], J. BALCITA-PEDICINO[1], J. WANG[1,2], Y. DONG[1]; [1]Neurosci., Univ. of Pittsburgh, Pittsburgh, PA; [2]Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Glutamate's importance as an excitatory neurotransmitter requires multiple regulatory mechanisms to control extracellular levels and plasticity changes associated with learning and memory. Astrocytes represent a major factor in regulating glutamate through expression of the main glutamate transporter and cysteine-glutamate exchanger. Maintenance of normal glutamate levels within the nucleus accumbens contributes to adaptive goal-directed behavior, a function compromised by exposure to psychostimulants like cocaine that disrupt astrocytic glutamate homeostasis. We sought to investigate potential morphological changes in astrocytes surrounding glutamate synapses within the nucleus accumbens core that might underlie the physiological and behavioral alterations identified in cocaine seeking. We also directly compared astrocytic associations with glutamate synapses in naïve rats versus the saline animals used as controls for cocaine self-administration. Adult male rats were trained to self-administer cocaine or saline 2 hours a day for 5 days and then sacrificed by intracardial perfusion with fixative 24 hours after the last session. Serial brain sections through the nucleus accumbens core were imaged by electron microscopy from cocaine self-administering, saline-treated, and naïve rats. *Reconstruct*^{TM} was used to generate 3-dimensional reconstructions of excitatory type axospinous synapses and nearby astrocytes. Within each rat group, extensive variation was observed in the degree to which astrocytes contacted glutamate synapses on spines. No significant differences were observed between cocaine (n = 77 synapses in 2 rats) and saline-treated animals (n = 72 synapses in 2 rats) in: 1) the size of axons, spines or synapses, 2) the extent to which astrocytes contacted glutamate synapses, or 3) the shortest extracellular distance between the active zone and the nearest astrocyte. We did, however, find significantly reduced mean spine and synapse volumes with overall lower variance in the saline-treated versus naïve animals (n = 166 synapses in 6 rats). Similar reductions were seen in cocaine self-administering rats, and these outcomes matched qualitative observations of an increased number of small spines in both treated groups. These results are consistent with the induction of spinogenesis, primarily by exposure to an enriched environment as opposed to drug. The short duration of self-administration might be a factor in this outcome. Additional animals are needed to improve statistical power in testing potential differences in astrocytic coverage of glutamate synapses between cocaine and saline self-administering animals.

Disclosures: S. Sesack: None. **T. Plute:** None. **J. Balcita-Pedicino:** None. **J. Wang:** None. **Y. Dong:** None.

Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA047269

NIH Grant DA035217

Title: Ibudilast attenuates cocaine self-administration and prime- and cue-induced reinstatement of cocaine-seeking rats

Authors: *X. LIU[1], L. MU[1], H. YU[1], M. HU[1], V. FRIEDMAN[1], L. ZHAO[2], Q.-S. LIU[1];
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Abstract: Ibudilast is a non-selective phosphodiesterase (PDE) inhibitor and glia cell modulator which has been used clinically to treat asthma and other inflammatory diseases. It has shown great promise for the treatment of drug and alcohol use disorders in recent clinical studies, but it is unknown whether and how ibudilast affects cocaine seeking behavior. Here we show that ibudilast pretreatments reduced cocaine self-administration under fixed- and progressive-ratio reinforcement schedules, and cocaine prime- and cue-induced reinstatement of cocaine seeking. However, ibudilast did not significantly affect sucrose self-administration. These results indicate that ibudilast was effective in reducing the reinforcing effects of cocaine and relapse to cocaine seeking without altering motivation to pursue and consume a natural reward. Chronic cocaine exposure induces cAMP-related neuroadaptations in the reward circuitry of the brain. To investigate potential mechanisms for ibudilast-induced attenuation of cocaine seeking, we recorded from ventral tegmental area (VTA) dopamine neurons in midbrain slices *ex vivo*. We found there was a decrease in inhibitory postsynaptic currents (IPSCs), an increase in the AMPAR/NMDAR ratio, and an increase in the excitation to inhibition (E/I) ratio. Ibudilast pretreatments enhanced GABAergic inhibition but did alter cocaine-induced potentiation of excitation, leading to normalization of the E/I ratio. In addition, ibudilast increased D_2 autoreceptor-mediated inhibition of VTA dopamine neurons. Thus, the restoration of the balance between excitation and inhibition and enhancement of D_2 autoreceptor-mediated inhibition in VTA dopamine neurons might contribute to the attenuation of cocaine seeking and relapse by ibudilast.

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Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA044749
NIH Grant DA045743

Title: Chronic cocaine craving and cue-elicited activity of the left middle/inferior frontal gyrus and skin conductance response

Authors: *C.-S. R. LI[1], S. ZHANG[2], W. WANG[3], S. ZHORNITSKY[1], T. M. LE[3], Y. CHEN[1], S. CHAUDHARY[1];
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Abstract: Background: Previous studies have examined cue reactivity as a behavioral and neural marker of cocaine addiction. Cocaine vs. non-drug cues elicited cortical and subcortical activities and some of regional responses may predict clinical outcomes. It is posited that drug cues are highly arousing and may elicit contextual memory that conduces to craving and drug seeking. However, no studies have specifically examined how cocaine vs. non-drug cues elicit changes in physiological arousal or how regional brain responses to these changes relate to chronic cocaine craving. **Methods:** We examined this issue in 39 cocaine users (CU) who met DSM criteria of a cocaine use disorder. During fMRI CU were exposed to cocaine and non-drug cues in alternative blocks. Skin conductance was recorded via a Biopac system throughout the experiment (Zhang et al., 2021, Biological Psychiatry: Global Open Science). We followed published routines in imaging and skin conductance data analyses and evaluated the results at a corrected threshold. **Results:** CU showed higher activation in fronto-parietal, visual, ventromedial prefrontal and mid-cingulate cortices as well as the striatum and midbrain, and lower activation in bilateral hippocampi during cocaine vs. nondrug blocks (Wang et al., 2021, Neuropsychopharm. 46: 851). Skin conductance response (SCR) showed a gradual decline over the 36-s block period but significantly less so during cocaine than during nondrug cue blocks. With the differences in SCR over the last 12 s as a regressor and age, sex, years of smoking and drinking as covariates, whole-brain regression identified activities in the left middle/inferior frontal gyri (M/IFG) in positive correlation with the SCR differences. Further, both M/IFG activities and SCR were significantly correlated with the severity of chronic cocaine craving, as assessed with the Cocaine Craving Questionnaire (CCQ) score. Mediation analyses showed that SCR completely mediated the correlation, bi-directionally, between CCQ score and M/IFG cue-elicited activities. **Conclusion:** These findings substantiate higher physiological arousal in response to cocaine vs. nondrug cues, M/IFG response as a neural marker of elevated physiological arousal, and a central role of physiological arousal in inter-relating cue-elicited prefrontal cortical activity and chronic cocaine craving. The roles of M/IFC in contextual memory and/or cognitive-emotional control during cue exposure remain to be investigated.

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Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH grant R00 DA038110

Title: Effects of sex and estrous cycle on cocaine-induced changes in excitatory synaptic transmission in the basolateral amygdala

Authors: *C. M. CORBETT[1,2], E. N. DUNN[1], B. D. ROOD[1], D. J. CHANDLER[1], J. A. LOWETH[1];
[1]Cell Biol. and Neurosci., Rowan Univ. Sch. of Osteo. Med., Stratford, NJ; [2]Rowan Univ. Grad. Sch. of Biomed. Sci., Stratford, NJ

Abstract: Sex differences in cocaine addiction liability and relapse vulnerability are influenced by fluctuations of ovarian hormones across the reproductive cycle. In rodent models in which cue-induced cocaine seeking increases or "incubates" during withdrawal from extended-access cocaine self-administration, females in the estrus stage of the estrous cycle (Estrus Females) show an increase in the magnitude of incubated craving compared to both males and females in all other stages of the cycle (Non-Estrus Females). However, the cellular and synaptic mechanisms contributing to these sex differences are unknown. One region known to play a critical role in the expression of incubated cocaine craving is the basolateral amygdala (BLA). We have previously reported an increase in the spontaneous firing rate of glutamatergic pyramidal neurons in the BLA in male rats during early withdrawal (2-3 weeks) from extended-access cocaine self-administration which correlates with changes in the magnitude of incubated cue-induced cocaine craving. Here we test the hypothesis that sex differences in incubated craving may be due, in part, to changes in BLA excitatory synaptic transmission. *Ex vivo* whole-cell patch clamp recordings from BLA pyramidal neurons were conducted following 2-3 weeks of withdrawal from extended-access cocaine self-administration or drug-naive conditions in adult male and female rats. Our preliminary data from male rats show a significant increase in spontaneous excitatory postsynaptic current (sEPSC) amplitude but no change in sEPSC frequency in cocaine-exposed rats compared to drug-naïve controls, suggestive of postsynaptic changes in excitatory transmission. Patch clamp recordings of BLA pyramidal neurons from cocaine-exposed females showed an increase in sEPSC frequency but not amplitude in Estrus compared to Non-Estrus Females, suggestive of presynaptic changes in glutamatergic transmission. To determine whether effects observed across the estrous cycle are estrogen-dependent, hormonal depletion and replacement studies were performed. Patch clamp recordings conducted in ovariectomized animals undergoing 2-3 weeks withdrawal from extended-access cocaine self-administration revealed an increase in sEPSC frequency but not amplitude in animals receiving chronic estradiol compared to vehicle-treated controls, mimicking effects observed in cocaine-exposed Estrus Females. Together our data indicate sex differences in BLA excitatory synaptic transmission during cocaine withdrawal that are influenced by estrous cycle fluctuations and that may contribute to known sex differences in incubated cocaine craving.

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Poster

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Program #/Poster #: P752.10

Topic: G.09. Drugs of Abuse and Addiction

Title: Adolescent Social Isolation Disrupts Developmental Profiles in Males and Females

Authors: *A. A. MILIAN, C. J. BENDERSKY, E. WOO, D. M. WALKER;
Behavioral Neurosci., Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: Adolescence is a crucial developmental period in which neural systems associated with motivation are particularly sensitive to rewarding and stressful stimuli. Social isolation (SI) during adolescence has been shown to result in long-term behavioral changes associated with anxiety and reward in adulthood in both males and females. Specifically, it has previously been reported that SI results in opposite effects on cocaine CPP and marble burying behaviors in male and female mice. However, little is known regarding how adolescent social isolation may disrupt somatic markers of development as well as development of specific reward and motivation-associated brain regions. Here we tested the hypothesis that adolescent social isolation disrupts developmental parameters and excitatory and inhibitory signaling within the reward circuitry which culminates in disruptions of sex-specific behaviors in adulthood. Mice were bred in-house and litters were weaned into either group housed or isolation housed mixed litter groups. After 3 weeks of isolation, around postnatal (P) 42, animals were re-socialized and housed 5 per cage until animals reached adulthood. To determine how SI disrupts development, the timing of puberty was monitored beginning on around P22 and animals were weighed every 10 days. Animals were euthanized either 4-6 hours after isolation, 10 days post-isolation, 4-6 hours after rehousing, or on P72, with brains collected for gene expression at each time point. Finally, previously published transcriptomic data was mined to determine if GABA and glutamatergic gene expression is disrupted by adolescent SI in reward-associated brain regions. Preliminary analysis revealed that SI advances the timing in puberty in males and females. While no effects on body weight were observed in females, isolated males were slightly larger than their control counterparts on P22 and P32 but weighed less than group housed males after rehousing. In adulthood, SI disrupted marble burying, elevated plus maze and cocaine CPP in a sex-specific manner. Mining of previously published transcriptomic data from the prefrontal cortex revealed sex-specific regulation of GABAergic receptor expression by cocaine after SI but had no effects on glutamate receptor expression. These data suggest that adolescent social isolation disrupts sex-specific development, behavior and gene expression. Current work is investigating how developmental profiles of glutamatergic and GABAergic related genes are disrupted by SI in the reward circuitry.

Disclosures: A.A. Milian: None. **C.J. Bendersky:** None. **E. Woo:** None. **D.M. Walker:** None.

Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: T32DK098107
NIH R01DA038613

Title: Mitochondrial gene ontology pathways and transcriptional regulators impacted by cocaine self-administration in C57Bl/6 mice

Authors: *C. A. CALARCO, S. M. KEPPETIPOLA, M. ENGELN, M. LOBO;
Anat. and Neurobio., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Exposure to illicit drugs and subsequent chronic use profoundly impacts behavior, neuronal structure and firing, and gene expression through multiple brain regions. Some of these sweeping changes are mediated by and supported by changes in cellular energy homeostasis and mitochondrial function. Recent work has shown that cocaine self-administration significantly alters mitochondrial size in nucleus accumbens medium spiny neurons, and that disruption of proper mitochondrial fission is sufficient to blunt responding for cocaine. Beyond mitochondrial fission, other aspects of the mitochondrial energy cycle are impacted by cocaine exposure. At the transcriptional level, many genes altered by cocaine self-administration are related to cellular metabolism and mitochondrial function. In this study we conducted gene ontology analysis on an RNA-seq data set generated from mice that had undergone cocaine self-administration, with a focus on mitochondrial-related ontology terms. The sequencing data set included tissue from the prefrontal cortex, nucleus accumbens, dorsal striatum, ventral pallidum, amygdala, hippocampus, and ventral tegmental area. We found significant representation of transcriptionally regulated genes in metabolism and mitochondrial related gene ontology terms in multiple brain regions. Further, predictive analysis of transcription factor regulation of identified mitochondrial-related genes identified a number of transcription factors across brain regions that may control cocaine-related changes in mitochondrial function. Future work will be needed to validate these predicted targets *in vivo* and understand their role in regulating the cellular and behavioral responses to cocaine self-administration.

Disclosures: C.A. Calarco: None. **S.M. Keppetipola:** None. **M. Engeln:** None. **M. Lobo:** None.

Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH R21DA046227

Title: Cell Subtype Transcriptional Role of Nab2 in Cocaine Seeking Behavior

Authors: *E. CHOI[1,2], R. CHANDRA[1], M. MCGLINCY[1], A. CHOW[1], M. LOBO[1];
[1]Dept. of Anat. & Neurobio., [2]Grad. Program in Life Sciences, Mol. Biol. & Biochem., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Substance use disorder is a debilitating chronic disease which is a leading cause of disability around the world. Drug seeking after repeated drug exposure is prevalent despite the devastating personal consequences. The nucleus accumbens (NAc, a.k.a. ventral striatum) is a major brain hub that mediates reward behavior. The NAc plays a role in drug seeking and addiction related behaviors in response to drug exposure. Studies have shown that exposure to psychostimulants, such as cocaine, is associated with molecular and functional imbalance in the two medium spiny neuron subtypes (MSNs) in the NAc, the dopamine receptor 1 and 2 enriched MSNs (D1-MSNs and D2-MSNs). While the study of D1-MSNs in the NAc with exposure to cocaine has been and is being extensively done, molecular changes and functional imbalance in the D2-MSNs remain largely elusive. We have previously reported the down regulated expression of a transcription factor, early growth response 3 (Egr3), in D2-MSNs in the NAc with exposure to cocaine. Furthermore, mice with Egr3 overexpression in D2-MSNs in the NAc showed altered extinction of cocaine seeking behavior after forced abstinence from cocaine self-administration. In this study, we show that the NGFI-A binding protein 2 (Nab2), corepressor of Egr3, is altered in bidirectional manner to Egr3 expression in D2-MSNs of mice repeatedly exposed to cocaine. Furthermore, we show that Nab2 overexpression leads to decreased levels of Egr3, and Nab2 inhibition leads to increased levels of Egr3. A2A-Cre mice with D2-MSN specific knock-down of Nab2 in the NAc showed decreased cocaine reduced locomotion. We then tested if Nab2 perturbation in D2-MSNs has a behavioral impact in cocaine self-administration. A2A-Cre mice with D2-MSN specific knockdown of Nab2 in the NAc showed reduced cocaine seeking after 10 days of cocaine self-administration. Our future plan is to study the brain tissue collected from these animals to elucidate transcriptomic changes occurring in selective cell types in the NAc via single nuclei RNA-seq. Collectively, our studies identify distinct cell type specific molecular mechanisms involved in behavioral responses to cocaine including drug seeking behavior.

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Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA–K99DA042100

Title: Adolescent social isolation stress and cocaine exposure disrupt sex-specific reward-related behavior and dopaminergic transcriptional programs in the medial amygdala of adult mice

Authors: *N. FOWLER, A. MILIAN, *C. BENDERSKY, D. WALKER;
Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: Adolescence is associated with the onset of sex-specific drug-reward-related behaviors and an increased sensitivity to reward and stress. Given dopamine's role in drug reward, transcriptional alterations within the mesocorticolimbic dopaminergic system may play a role. Most studies that have examined this system have focused on brain regions already implicated in reward (e.g., nucleus accumbens). However, the medial amygdala (meAMY), a region traditionally thought to be outside of the canonical reward circuitry, is also rich in dopamine receptors and plays a crucial role regulating sex-specific drug reward during adolescence. Despite this, little is known about how adolescent stress or drug exposure influence dopaminergic transcriptional programs in the meAMY in a sex-specific manner. Therefore, this study examined dopaminergic gene expression in the meAMY of adult female and male mice following adolescent social isolation stress and/or cocaine exposure. Female and male mice were exposed to social isolation stress or group housed during adolescence (P22-P42) and then group housed until adulthood (P90). In adulthood, animals underwent behavioral testing in novelty suppressed feeding and cocaine CPP to measure animals response to a novel and rewarding stimulus. Additionally, publicly available transcriptomic data were mined for gene expression changes associated with dopaminergic receptor expressing neurons after the first dose of cocaine in males and females (Walker et al., 2020). Sex differences in novelty suppressed feeding were lost after adolescent social isolation and a previously described sex-specific effect on cocaine CPP was observed. In addition to baseline sex differences in dopaminergic Drd1 gene expression in the meAMY of adult mice (female > male), we found that sex-specific expression was lost following adolescent social isolation stress. We also found that cocaine only induced expression of Drd1 and Drd2 associated genes in isolated males. These data suggest that adolescent isolation disrupts adult sex-specific behavior as well as drug-induced dopaminergic transcriptional programs in the meAMY which may promote long-lasting synaptic and behavioral adaptations associated with addictive-like behaviors. Current work is focused on investigating how adolescent isolation disrupts developmental profiles of Drd1 and Drd2 expression.

Disclosures: N. Fowler: None. **A. Milian:** None. **C. Bendersky:** None. **D. Walker:** None.

Poster

P752. Cocaine: Cellular and Neurophysiological Mechanisms

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P752.14

Topic: G.09. Drugs of Abuse and Addiction

Support: National Institute of General Medical Sciences (GM084854)
National Center for Research Resources (5R25GM061838-15, 2G12-RR003051)
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NSF Partnerships in International Research and Education (PIRE) Program Neural Mechanisms of Reward & Decision (OISE-1545803)
Research Initiative for Scientific Enhancement RISE Program (5R25GM061151-18)
Centre National de la Recherche Scientifique (CNRS), the Universite de Bordeaux and by LABEX BRAIN ANR-10-LABX-43

Title: Progressive hyperpolarization-activated cation current (I_h) reduction: a response mechanism to decrease cocaine-induced excitability in VTA DA neurons.

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Abstract: The hyperpolarization-activated cation current (I_h) is a contributing factor of intrinsic neuronal excitability in different cells, including dopaminergic neurons (DA) of the ventral tegmental area (VTA). In contrast to other cellular conductances, I_h is activated by hyperpolarization and produces a time-dependent depolarizing current. Our laboratory demonstrated that cocaine sensitization, a non-contingent cocaine model, significantly reduces I_h amplitude in VTA DA neurons. However, the role of I_h in controlling VTA DA excitability is still poorly understood. It has been shown that VTA DA cell's spontaneous activity remains unchanged when compared to the saline control groups after cocaine sensitization. Our hypothesis is that I_h reduction could function as a homeostatic regulator compensating for the cocaine-induced alteration in excitability thus, explaining the above-mentioned results. Using *in vivo* single-unit extracellular electrophysiology in isoflurane-anesthetized rats, we explored the Ih contribution on acute cocaine-induced VTA neuronal spontaneous activity. We locally perfused an I_h blocker (ZD7288, 8.3 μM) and evaluated its effect on VTA DA spontaneous firing

patterns. I_h blockade significantly reduced acute cocaine-induced spontaneous firing rate, bursting frequency, and percent of spikes within a burst. I_h local inhibition also significantly increased the mean interspike interval (ISI) in VTA DA neurons. These findings suggest that I_h blockade can significantly reduce cocaine-induced firing activity in VTA DA neurons in part due to an increase in the mean ISI. Furthermore, using whole-cell patch-clamp, we found a significant reduction in I_h after 24 but not 2hours (hrs) of acute cocaine administration (15mg/kg, i.p). Using a current clamp rebound spiking protocol, we determined at different intervals of acute cocaine administration (2hrs, 24hrs and, 24hrs following 7-days cocaine administration) that there was a significant increase in rebound action potentials. This increase was progressively diminished as the sensitization protocol developed. In addition, the I_h blockade significantly reduced rebound action potentials. These results suggest that a progressive Ih reduction could serve as a homeostatic regulator of cocaine-induced spontaneous firing patterns which are correlated to an enhanced VTA DA excitability.

Disclosures: K.Y. Bosque Cordero: None. **R. Vazquez-Torres:** None. **C. Calo-Guadalupe:** None. **D. Consuegra-Garcia:** None. **G.R. Fois:** None. **F. Georges:** None. **C.A. Jimenez-Rivera:** None.

Poster

P951. Theoretical Network Models I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P951.01

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

Support: NIMH109548

Title: Cross-frequency coupling in a macroscopic model of collective neural activity

Authors: *A. SHEREMET[1], Y. QIN[2];
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Abstract: Collective neural activity, i.e., spatiotemporal patterns of activity that entrain a large subset of the neural population, such as oscillations, propagating waves, Turing patterns, spiral waves and others, is macroscopic with respect to cell scale, in the sense that the details of the behavior of any single cell have no detectable expression in its dynamics. In physics, the evolution of large scale systems has been traditionally modeled in the framework of statistical physics, or its thermo-/hydrodynamic limits, which represent the physical system as a continuum. These approaches have been successfully used to investigate phenomena such as phase transitions, that bear little resemblance to microscopic dynamics.
The application of such models to brain activity, however, poses a fundamental challenge: there are no neurons, as "cell individual", in these models. These models do not describe pairwise

communication between neurons, or deterministic interconnections between small groups of neurons. If brain activity depends crucially on the dynamics of cell individuals, rather than populations, these models might not be of much use.
However, measurements reveal intriguing macroscopic features of brain activity such as strong nonlinear deformation of the theta rhythm in rats running at high speed, cross-frequency coupling between theta and gamma rhythms, and cross-scale coupling in transient processes such as sharp waves and ripples, or slow waves and spindles. These features are similar to those observed in large scale systems such as wave turbulence, where they have been successfully studied using statistical models. Here, we present an investigation into the properties of a macroscopic model of collective activity and demonstrate that cross-frequency coupling is a fundamental phenomenon that originates in the stochastic and nonlinear character of macroscopic dynamics.

Disclosures: A. Sheremet: None. **Y. Qin:** None.

Poster

P951. Theoretical Network Models I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P951.02

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

Support: NIMH109548

Title: A macroscopic formulation of collective neural activity based on the Hodgkin-Huxley model

Authors: *Y. QIN[1], A. SHEREMET[2];
[2]Engin. Sch. for Sustainable Infrastructure and Envrn. (ESSIE), [1]Univ. of Florida, Gainesville, FL

Abstract: Collective activity is defined by the emergence of spatiotemporal patterns of activity that entrain a large subset of the neural population, such as oscillations, propagating waves, Turing patterns, spiral waves and others. Collective activity is macroscopic with respect to the cell size, in the sense that many details of cell-scale dynamics, such as the exact shape of the action potential of any given single cell, are ignorable. In the cortex, collective activity is mesoscopic, with characteristic scales of 0.1 - 10 mm and 5 - 500 ms. While one could conceivably attempt to model collective activity by integrating a cell-scale model (e.g., the Hodgkin-Huxley equations) for every participating neuron, the large number of cells involved and the lack of accurate information about the individual cells and the structure and characteristics of the interneuronal connections, make this an impossible task. It is also an unnecessary task, because the details of the behavior of a single cell have no detectable

expression in collective activity. In physics, the evolution of large scale systems has been traditionally modeled in the framework of statistical physics, or its thermo-/hydrodynamic limits, which represent the system as a continuum. This approach was used successfully to explain phenomena such as phase transitions, that are fundamentally macroscopic in nature and bear little resemblance to microscopic dynamics.
Here, we present a statistical formulation of collective activity dynamics and explore further macroscopic simplifications. Starting from the dynamical equations of Hodgkin and Huxley, we formulate a Fokker-Plank-type statistical model describing evolution of the distribution of the neural population in the parameter space of the Hodgkin-Huxley model. In the long-wave limit we reduce the dimensionality of the Fokker-Plank representation to a small number of state variables that are observable at macroscopic scale (e.g., through LFP measurements). The resulting model is similar in functionality to a hydrodynamic description of gas. We compare the model with other existing formulations of the same type (e.g., Wilson-Cowan models), and present a preliminary analysis of the linear properties of the model.

Disclosures: Y. Qin: None. **A. Sheremet:** None.

Poster

P951. Theoretical Network Models I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P951.03

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

Title: Mechanisms underlying non-deterministic computation in cortical neural networks

Authors: *E. A. STOLL;
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Abstract: Unlike peripheral neurons, which have highly deterministic signaling outcomes, cortical neurons exhibit probabilistic firing patterns, permitting random electrical noise to gate signaling outcomes. While statistical tools have proven successful at modeling probabilistic firing patterns in the cortex, there is no established mechanism for computational units to achieve optimal coding by relying on stochastic membrane potential fluctuations. Here, a mechanistic process is proposed to explain how neurons achieve non-deterministic signaling outcomes during up-state, by retaining sensitivity to stochastic charge flux and using this noise to drive computational work. Here, the voltage state of each neuron is modeled as a mixed sum of pure states, accounting for the relative position and atomic orbital of every local electron after some time evolution. This mixed sum of component states is a complex-valued probability density, representing the total information or von Neumann entropy of the system. The probability amplitudes constructively and destructively interfere in a physical manner on the complex plane to yield a non-deterministic computational outcome for each neuron in the network. The quantity

of energy expended to create information is partially recovered during a computational cycle, as predictive value is extracted; that quantity of predictive value is equivalent to the quantity of information compression and the quantity of free energy released back into the system. This model demonstrates how cortical neurons can temporally bind and parse information to extract predictive value, undergoing cycles of information generation and compression to compute the most likely state of the local environment.

Disclosures: E.A. Stoll: None.

Poster

P951. Theoretical Network Models I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P951.04

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

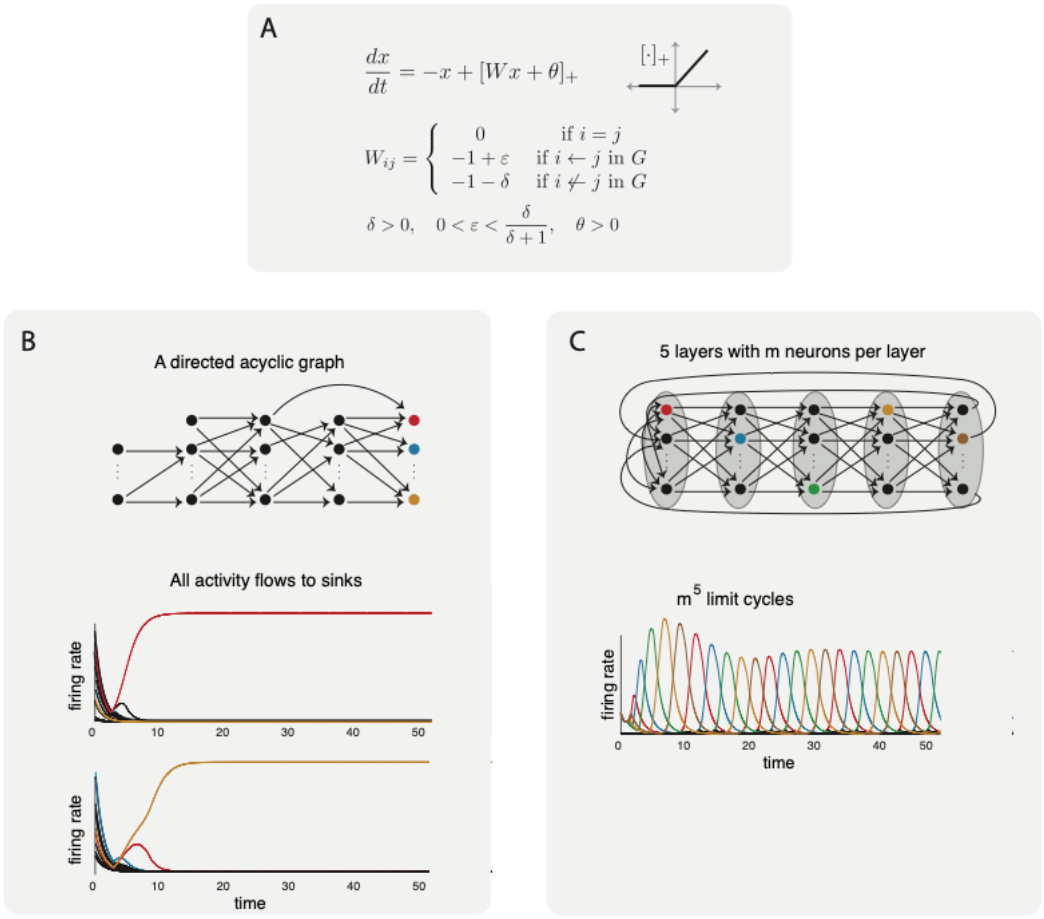
Support: NIH R01 NS120581
NSF DMS-1951165
NIH R01 EB022862
NSF DGE1255832

Title: from structure to dynamics in combinatorial threshold linear networks

Authors: *C. LIENKAEMPER[1], K. MORRISON[2], C. P. CURTO[3];
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Abstract: Neural circuits display nonlinear dynamics. The structure of network connectivity is a key feature determining network dynamics, but many questions remain as to how structure shapes activity. We study this relationship in a simple model of neural activity, combinatorial threshold linear networks (CTLNs), whose activity is governed by a system of threshold-linear ordinary differential equations determined by a directed graph (Figure A). CTLNs display the full spectrum of nonlinear behavior, including multistability, limit cycles, and chaos.
Much is known about fixed points of CTLNs, but much less is known about their dynamic attractors. Here we go beyond fixed points and relate the structure of a CTLN to its dynamics. We focus on a structural relationship, graphical domination, and show that if one neuron graphically dominates another, then the firing rate of the dominating neuron eventually becomes greater than the firing rate of the dominated neuron. This constrains trajectories of the dynamical system. Using this fact, we show that many CTLNs have only static (fixed point) attractors. Specifically, we prove that if a CTLN's underlying graph is a directed acyclic graph, neural activity must converge to a stable fixed point supported on one of the sinks (Figure B). This type of convergence result was previously only known in the case of symmetric TLNs.

We also construct a family of sequential memory networks. Each network consists of m layers of n neurons connected cyclically (Figure C). The network has m^n limit cycles. Different initial conditions lead to different limit cycles (Figure C). Our domination result allows the network to "remember its place" once it comes back around to a previously visited layer. These networks have a large capacity to encode dynamic patterns via limit cycles, giving a richer set of memory patterns than stable fixed points. Thus, these networks can model sequential memories, rhythms, or central pattern generators.



Disclosures: C. Lienkaemper: None. **K. Morrison:** None. **C.P. Curto:** None.

Poster

P951. Theoretical Network Models I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P951.05

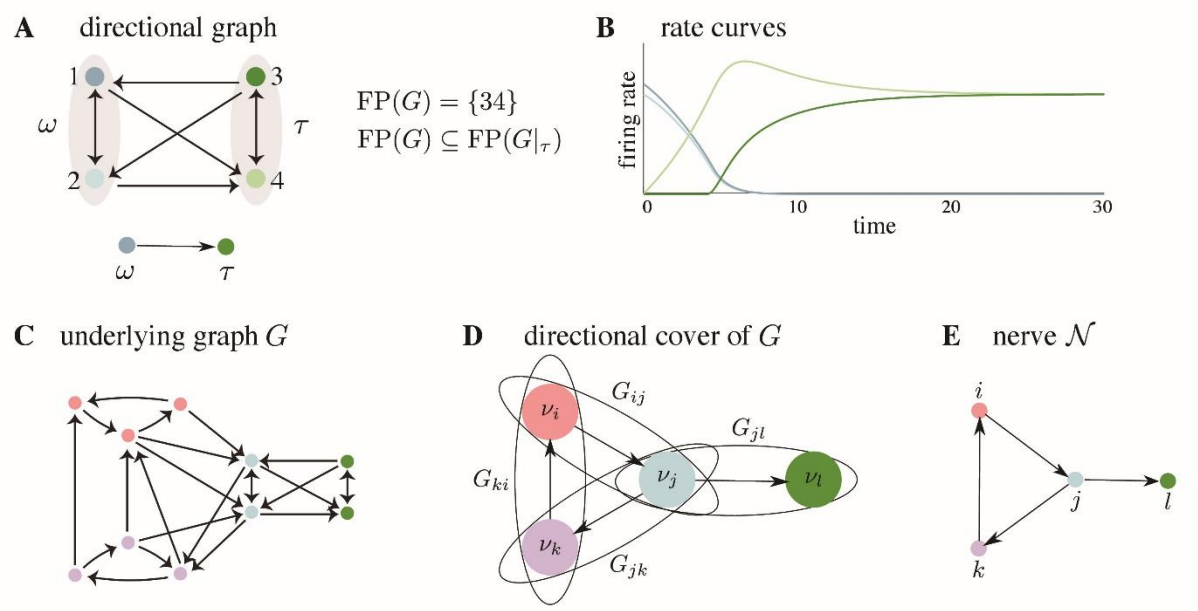
Topic: I.06. Computation, Modeling, and Simulation

Support: NIH R01 NS120581
NSF DMS-1951165
NSF-CCF 1841455
NSF-HRD 1500481

Title: Nerve theorems for dimensional reduction of threshold-linear networks

Authors: *N. SANDERSON[1], D. EGAS SANTANDER[3], S. EBLI[3], A. PATANIA[4], F. BURTSCHER[5], K. MORRISON[6], C. P. CURTO[2];
[2]Mathematics, [1]The Pennsylvania State Univ., University Park, PA; [3]E´cole Polytechnique F´ed´erale de Lausanne, Lausanne, Switzerland; [4]Indiana Univ. Network Sci. Inst. (IUNI), Bloomington, IN; [5]Universit´e du Luxembourg, Belvaux, Luxembourg; [6]Univ. of Northern Colorado, Greeley, CO

Abstract: Combinatorial threshold-linear networks (CTLNs) are a mathematically tractable subfamily of threshold-linear networks whose dynamics are determined by the structure of a directed graph. Threshold-linear networks have been widely used to model neural networks with associative memory. ln spite of their simplicity, CTLNs exhibit the full range of nonlinear dynamics: multistability, limit cycles, quasiperiodic and chaotic attractors. One of the most remarkable features of CTLNs is the strong relationship between the attractors of the dynamics and the collection of fixed points. Critically, these fixed points can often be completely determined by the structure of the graph. When the fixed points are confined to a subset of the neurons, neural activity has been observed to converge to this subset. In this sense, attractors live where the fixed points live. This motivates the idea of directional graphs, networks that exhibit feedforward dynamics without requiring a feedforward architecture, as a proxy for the expected flow of activity. Specifically, any subnetwork that is a directional graph acts like a single directed edge in terms of the flow of activity through the larger network. We find that whenever a large network can be covered by directional graphs, then we can associate a simplified nerve graph where each directional subnetwork is replaced by a directed edge. Many properties of the larger network can then be understood in terms of those of the nerve graph, resulting in a dramatic dimensionality reduction. We will present a number of results relating the fixed points of the nerve graph to those of the larger network. We will also show examples of how to engineer complicated network architectures with desired dynamic properties and how to investigate the dynamics of complex networks using this powerful dimensionality reduction technique.



Disclosures: N. Sanderson: None. **D. Egas Santander:** None. **S. Ebli:** None. **A. Patania:** None. **F. Burtscher:** None. **K. Morrison:** None. **C.P. Curto:** None.

Poster

P951. Theoretical Network Models I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P951.06

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

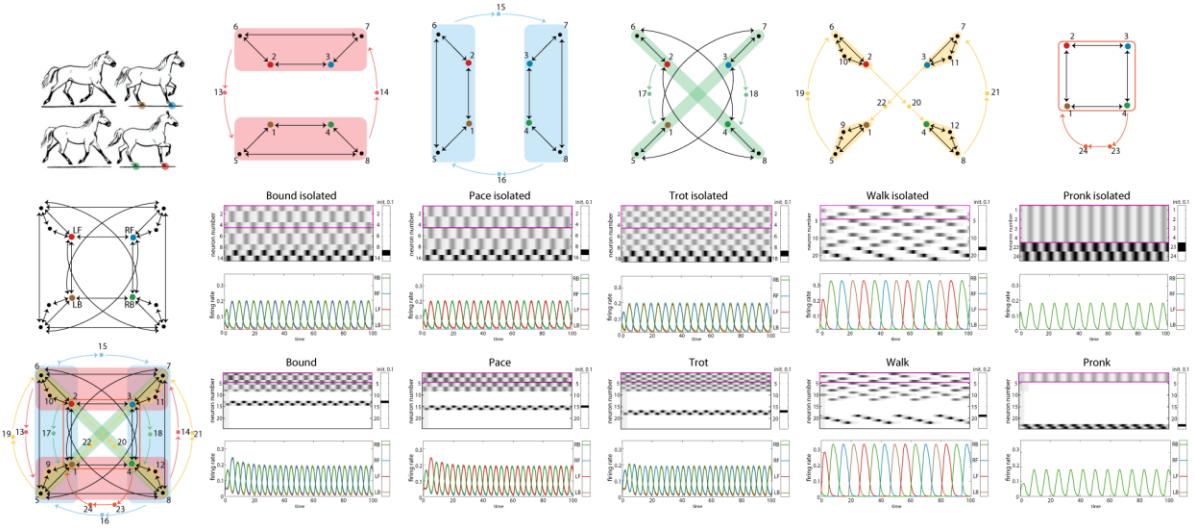
Support: NIH R01 NS120581
NSF DMS-1951165

Title: Combinatorial threshold linear networks: a unifying framework for temporal pattern generation.

Authors: *J. LONDONO ALVAREZ[1], K. MORRISON[2], C. P. CURTO[1];
[1]Mathematics, The Pennsylvania State Univ., University Park, PA; [2]Sch. of Mathematical Sci., Univ. of Northern Colorado, Greeley, CO

Abstract: Threshold-linear networks (TLNs) are widely used as a framework for modeling recurrent neural networks, including associative memory networks. Here we focus on a special family of TLNs, known as Combinatorial Threshold Linear Networks (CTLNs), a firing rate model with binary synapses and simple perceptron-like neurons. The dynamics of CTLNs are controlled solely by the structure of a directed graph, thus isolating the role of connectivity in

network dynamics. Moreover, its piecewise-linear dynamics provide a mathematically tractable framework where all types of nonlinear dynamics can still arise (multistability, limit cycles, chaos, quasiperiodicity). This simple mathematical setup makes it suitable for engineering circuits performing common neural functions, yet still allowing flexibility in terms of the kind of dynamics that might be observed. Our aim here is to present a few interesting cases that exemplify how connectivity alone gives rise to a diverse range of neural functions. Our first example is a CTLN that can count the number of pulse inputs it has received via the position of the attractor in a linear chain of attractor states; this network is a simple yet robust alternative to the neural integrators typically used to maintain a count in working memory of some number of input cues. With a small variation in the previous network, we can obtain a signed counter, where the dynamics can now travel back to the previous attractor on the chain as well. This proves valuable for tracking the relative number of left and right cues as in various two-alternative forced-choice tasks or tracking position in a linear track. Finally, we present a CTLN capable of producing five coexisting quadrupedal gaits. These gaits coexist as distinct limit cycle attractors in the network, without needing to change parameters to access different gaits (Fig. 1). Instead, different gaits are obtained by changes in initial conditions, or by stimulation of a specific neuron involved in the gait. These examples illustrate the power of CTLNs as a unifying framework for modeling a wide range of neural computations.



Disclosures: J. Londono Alvarez: None. **K. Morrison:** None. **C.P. Curto:** None.

Poster

P951. Theoretical Network Models I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P951.07

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

Support: Sloan Research Fellowship

Title: Sparse expander graph connections for efficient long-range cortical information flow and distributed working memory

Authors: *A. DE, R. CHAUDHURI;
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Abstract: Long-range connections in the cortex are sparse. For example, the number of connections in the primate neocortex falls off exponentially with distance (Ercsey-Ravasz et al., 2013). How could distant cortical circuits effectively communicate given these extremely sparse interactions? To address this question, we draw on recent mathematical results in the theory of expander graphs. Expander graphs are sparse networks that are well-connected despite their sparsity. They have found widespread recent use in computer science in designing powerful algorithms for efficient communication, error correction, dimensionality reduction, and sparse signal recovery. Moreover, large, sparse, random networks are expander graphs thus making them attractive candidates for modeling biological connectivity.
We consider a model of two interacting brain areas, connected by sparse expander connections. We first show that a small number of such connections between the source and target area is sufficient to preserve information about sparse patterns in the source area, thus allowing these input patterns to be efficiently communicated to the target area. Next, we show how such sparse connections can be used to sense and reconstruct sparse sensory input patterns using simple neurally-plausible dynamics and only a small number of reconstruction neurons ("compressed sensing"). Importantly, the sparsity of the connections is essential in allowing errors to be identified and the signal to be reconstructed using simple dynamics. We show that, among other uses, such sparse signal reconstruction could be used as part of a sensory-cognitive loop to maintain stimulus-specific persistent activity for a wide range of stimuli, providing theoretical support for recent models of flexible distributed working memory (Bouchacourt & Buschman, 2019). Finally, we investigate when such sparse connections could allow a control signal in one area to efficiently shape neural dynamics and computation in the other area, as could be done for information gating and cognitive control.
Our results provide a novel mathematical framework in which to model sparse long-range interactions in the cortex, with possible applications to efficient information flow, error correction, distributed working memory, and cognitive control.

Disclosures: A. De: None. **R. Chaudhuri:** None.

Poster

P951. Theoretical Network Models I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P951.08

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

Support: NIH Grant 5T90DA032435-10

Title: Multiple Spike Sequences Inherent in Random Networks

Authors: *H. GERMAINE, P. MILLER;
Brandeis Univ., Waltham, MA

Abstract: The mammalian hippocampus contains place cells which fire in a sequence representing a traversal through space. Interestingly, the same cells can represent different locations in different environment traversals, meaning spike sequences for unique environments can contain overlapping neuron populations. Further, research has shown that the trajectory-representing spike sequences can be observed prior to an animal ever experiencing an environment, pointing to an inherent tendency for sequences to exist and then be mapped onto environments upon experience. Computational models of these sequences traditionally employ a spatially organized lattice network of neurons, with spatially organized connectivity, representing a two-dimensional set of place fields in an environment. These models can accurately represent firing patterns in relation to one environment, but not multiple. In this study, we aim to test a non-spatially organized network organization for inherent spike sequences which are numerous and orthogonal. To do so, we utilize a leaky integrate-and-fire model of neuronal firing and create a neural network structure organized into randomized and overlapping clusters of neurons, within which connectivity is sparse and randomized. To mimic an environmental stimulus causing a sequence of firing, we set one cluster of neurons to threshold, and observe the trajectory of firing through the network. Using this model, we show that a non-spatially organized network of neurons is able to achieve unique sequences of firing representing different environmental trajectories, where individual neurons take part in multiple trajectories. This means that the set of neural spikes which follow the spiking of a particular neuron depends on the set of spikes which preceded its firing. Analysis using a matching index (MI) metric as well as Spearman's rank order correlation results in values of 0.19 ± 0.09 and 0.30 ± 0.10 respectively, indicating a low degree of similarity in the firing sequences, but overall supporting a strong differentiation for different initiations of sequences. We further present how Hebbian strengthening of one trajectory causes a deterioration of other trajectories. These results not only contribute to more biologically relevant modeling of hippocampal activity but also support the inherent tendency of networks to generate "preplay" sequences, which can then be mapped onto particular environments and strengthened through learning.

Disclosures: H. Germaine: None. **P. Miller:** None.

Poster

P951. Theoretical Network Models I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P951.09

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

Title: Spectral analysis of a recurrent circuit model implementing normalization

Authors: *S. RAWAT[1], D. J. HEEGER[2], S. MARTINIANI[1];
[1]Chem. Engin. and Materials Sci., Univ. of Minnesota, Twin Cities, Minneapolis, MN; [2]Dept Psychol & Ctr. Neural Sci., New York Univ., New York, NY

Abstract: Background: Divisive normalization (DN) is a canonical neural computation in which the response of a neuron is divided by the total activity of an ensemble of neurons. Experimental evidence suggests that DN operates via recurrent amplification. Several circuit models implementing DN have been proposed, but they do not rely on recurrent amplification to achieve DN and/or they do not exhibit oscillatory dynamics linked to normalization and/or they only approximate DN (which has practical consequences for making experimentally-testable predictions and for fitting data). Oscillatory Recurrent Gated Neural Integrator Circuits (ORGaNICs) are a recently proposed family of recurrent circuit models that implement DN via recurrent amplification. Methods: We implemented a multistage ORGaNICs model to simulate and study the dynamics of neural activity and communication across cortical hierarchies in the primate visual system. We derived closed-form expressions for the power spectral density and coherence of the responses within a local circuit and across multiple stages using the properties of continuous-time stochastic processes. Results: We found, in agreement with electrophysiological measurements in non-human primates, that: 1) the power spectrum of the local field potentials (LFPs) is shaped by recurrent normalization to exhibit a resonant peak in the gamma frequency range (~40-80 Hz); 2) the peak frequency and the amplitude of oscillatory activity are dependent on stimulus contrast; and 3) the shape of the LFP power spectra remains largely unchanged across cortical areas, except for a modest increase in the peak resonant frequency. Analysis of the coherence between stages reveals that inter-area communication is frequency and contrast dependent, so that coherence is maximized at low frequencies for low contrast stimuli, while coherence is maximized in the gamma-frequency range for high contrasts. Conclusion: Our theory offers a novel link between a local circuit-model of DN and large-scale neural dynamics.

Disclosures: S. Rawat: None. **D.J. Heeger:** None. **S. Martiniani:** None.

Poster

P983. All Optical Interrogation of Biological Systems

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P983.01

Topic: I.08. Methods to Modulate Neural Activity

Support: HFSP LONG-TERM FELLOWSHIP
SNF postdoc mobility
HHMI
NIH

Title: All-optical experiments across cortical areas enabled by novel transgenic mouse lines expressing the red-shifted opsin ChRmine

Authors: *A. DRINNENBERG[1], S. QUIRIN[1], C. RAMAKRISHNAN[1], T. L. DAIGLE[2], B. TASIC[3], L. SIVERTS[3], S. RANDLES[1], A. GERBINO[1], H. ZENG[4], K. DEISSEROTH[5]; [1]Stanford Univ., Palo Alto, CA; [3]Cell and Circuit Genet., [2]Allen Inst. For Brain Sci., Seattle, WA; [4]Structured Sci., Allen Inst. for Brain Sci., Seattle, WA; [5]Bioengin & Psych, Stanford Univ. Dept. of Psychology, Stanford, CA

Abstract: In all-optical experiments, the simultaneous readout and manipulation of neuronal activity is achieved by combining 2-photon calcium imaging with 2-photon holographic photostimulation. Experimental success of all-optical approaches depends on carefully optimized expression levels of both opsin and indicator to ensure high fidelity photostimulation and high SNR activity readout, with minimal crosstalk. Viral approaches have proven to be challenging to ensure well-titrated, stable, and widespread co-expression of opsin and indicator. Here, we present a transgenic approach for all-optical neuroscience. We developed four novel TIGRE-based transgenic reporter lines (*Ai219, Ai228, Ai229, Ai230*). All four lines are Cre-dependent and express the red-shifted, excitatory opsin ChRmine, targeted to the soma to facilitate single cell precision of the photostimulation. *Ai219* is a ChRmine-oScarlet reporter that can be combined with various indicators - either virally delivered, or by breeding to a second reporter line. *Ai228* is a GCaMP6m and ChRmine-oScarlet reporter that leads to high quality all-optical preparations with stable expression levels for >2 months. *Ai229* is a GCaMP6m and ChRmine reporter without fluorophore, adding further flexibility for cell-type or projection-specific labeling. *Ai230* co-expresses ChRmine-oScarlet and the fast indicator XCaMPG upon viral delivery of Cre and tTA. We demonstrate the utility of the four reporters for all-optical experiments by using various Cre delivery strategies. In *Ai219*, for example, we used a bicistronic AAV to deliver Cre and GCaMP6m to excitatory cortical neurons and achieved high fidelity photostimulation (>90% stimulation-responsive cells of all targeted cells, 5mW per cell). For all-optical access throughout cortex, we employed a full transgenic approach using *Ai228xCaMK2aCre-ERT2*, resulting in widespread and homogenous expression of ChRmine and GCaMP6m in excitatory neurons with virtually no neurons (<1%) having filled nuclei. Using 2nd generation multiSLM scopes, we performed all-optical experiments (FOV >1mm2) across areas of visual cortex, after defining area boundaries using retinotopic maps. From a pool of ~1500 neurons, we chose ensembles of up to 65 neurons for simultaneous stimulation and obtained a high fraction (>90%) of stimulation-responsive cells (at 6mW per cell), with the maximal distance between successfully targeted cells >1.5mm. Our suite of transgenic reporter lines for all-optical experiments can be flexibly combined with existing cell-type specific viral or transgenic targeting strategies, and offers new possibilities for all-optical neuroscience.

Disclosures: A. Drinnenberg: None. **S. Quirin:** None. **C. Ramakrishnan:** None. **T.L. Daigle:** None. **B. Tasic:** None. **L. Siverts:** None. **S. Randles:** None. **A. Gerbino:** None. **H. Zeng:** None. **K. Deisseroth:** None.

Poster

P983. All Optical Interrogation of Biological Systems

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:15 PM - 3:15 PM

Program #/Poster #: P983.02

Topic: I.08. Methods to Modulate Neural Activity

Support: HHMI
NIH Grant 1RF1MH117042-01

Title: All-optical electrophysiology with improved genetically encoded voltage indictors reveals interneuron network dynamics in vivo

Authors: *H. TIAN[1], H. C. DAVIS[1], D. WONG-CAMPOS[1], L. Z. FAN[2], B. GMEINER[1], Y. QI[1], P. PARK[1], V. PAROT[1], C. A. WERLEY[3], G. B. BORJA[3], S. BEGUM[1], H. UPADHYAY[3], H. SHAH[3], J. JACQUES[3], K. HARIKRISHNAN[3], K. DEISSEROTH[2], A. E. COHEN[1];
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Abstract: All-optical electrophysiology is a promising methodology for studying neural dynamics in vivo, as it offers the ability to image and perturb membrane voltage in multiple cells simultaneously. The “Optopatch” constructs combine a red-shifted archaerhodopsin (Arch)-derived genetically-encoded voltage indicator (GEVI) with a blue-shifted channelrhodopsin actuator (ChR). We used a video-based pooled screen to evolve Arch-derived GEVIs with improved signal-to-noise ratio (QuasAr6a) and kinetics (QuasAr6b). Both constructs enabled minutes-long continuous high-speed voltage imaging in vivo through multiple imaging sessions. Using an Optopatch construct based on QuasAr6a, we showed that optogenetically-triggered activation of a single Ndnf-expressing neuron in visual cortex may lead to a significant reduction of the spike rate in neighboring interneurons, in a distance-dependent manner. Using QuasAr6b-based Optopatch, we characterized the excitability of fast-spiking parvalbumin (PV)-expressing interneurons in hippocampus CA1. We further examined the spontaneous spiking of multiple PV neurons and discovered an increased probability of near-synchronous spikes in closely spaced cells. Together, these results demonstrate powerful new tools for all-optical microcircuit dissection in live mice.

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Harikrishnan: None. **K. Deisseroth:** None. **A.E. Cohen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Q-State Biosciences.

Poster

P983. All Optical Interrogation of Biological Systems

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Program #/Poster #: P983.03

Topic: I.08. Methods to Modulate Neural Activity

Support: National Science Foundation NeuroNex program
NOMIS Foundation
Else Kröner Fresenius Foundation
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Title: All-optical interrogation of excitatory-inhibitory network dynamics in vivo using red-shifted ChRmine channelrhodopsins created by structure-guided design

Authors: *M. INOUE[1], P. Y. WANG[1], Y. KIM[1], T. BENSTER[2], S. QUIRIN[1], C. RAMAKRISHNAN[1], M. LO[1], K. E. KISHI[5], M. FUKUDA[5], E. F. X. BYRNE[1], E. THADHANI[3], J. M. PAGGI[3], H. E. KATO[5], K. DEISSEROTH[4];
[1]Bioengineering, [2]Neurosci&Bioengin, [3]Computer Sci., [4]Bioengin & Psych, Stanford Univ., Stanford, CA; [5]Univ. of Tokyo, Tokyo, Japan

Abstract: Brain circuit architecture includes reciprocal connections between independent populations of neurons that are defined by their electrophysiological and transcriptional properties. To characterize the flow and transformation of information through these neural circuits, it will be important to achieve simultaneous measurement and perturbation of activity within distinct cell populations during behavior, and concurrently measuring and controlling cell type-specific neural activity using all-optical molecular tools is becoming a powerful relevant approach. While many microbial opsins have been discovered and engineered for optical control, current optogenetic tools for all-optical neuroscience are limited in speed and combinatorial spectral compatibility with activity sensors (e.g. GCaMP). To resolve this shortcoming and enable multiple-population all-optical neural circuit interrogation, we solved the cryo-EM structure of the channelrhodopsin ChRmine and created novel variants (designed based on the structure) including rsChRmine (which exhibits a reduced blue light shoulder and enhanced red light response, and responds to infrared light (750 nm) *in vivo*. Here, we have compared this new opsin to other class members and validate utility with both 1-photon and 2-photon implementation. Furthermore, we have combined this opsin with multiple genetically-encoded calcium indicators through frame-projected independent-fiber photometry to develop a novel

method for neural circuit interrogation termed ‘type-to-type synaptic weight’. This approach writes-in activity patterns to defined neural circuit elements and simultaneously reads-out the response of multiple defined cell types within the same network *in vivo*. Applying this approach to the mPFC of freely-behaving mice, we have identified quantitative dynamics of information transmission between feedforward and feedback circuitry in prefrontal excitatory and inhibitory networks that depended upon spike train length and frequency. These results establish an all-optical neural circuit interrogation approach for uncovering principles of interaction between cell types in the mammalian brain during behavior.

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Poster

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Program #/Poster #: P983.04

Topic: I.08. Methods to Modulate Neural Activity

Support: Helen Hay Whitney Foundation
HHMI

Title: All-optical electrophysiology of hippocampal plasticity

Authors: *L. Z. FAN[1], J. JENNINGS[1], H. TIAN[2], E. THADHANI[1], Y. SUN[1], E. KIM[1], S. RANDLES[1], P. WANG[1], C. RAMAKRISHANAN[1], Y. S. KIM[1], S. QUIRIN[1], V. HELM[1], A. E. COHEN[3], K. DEISSEROTH[1];
[1]Stanford Univ., Palo Alto, CA; [2]Dept. of Chem. and Chem. Biol., Harvard Univ., Cambridge, AL; [3]Dept. of Chem. and Chem. Biol., Harvard Univ., Cambridge, MA

Abstract: Learning and memory have been associated with changes in synaptic strength and circuit dynamics, yet the precise plasticity mechanisms naturally employed for information storage in the brain have remained largely unclear. Here we investigated plasticity mechanisms in the hippocampus in vivo during behavior by combining genetically-targeted voltage imaging with optogenetic activation. Voltage imaging in the head-fixed virtual-reality setting revealed physiological circuit properties and dynamics expected for mouse behavior, including complex spikes, theta oscillations, and place cells (n = 11 place cells out of 57 recorded spiking cells, N = 4 mice). We showed that targeted optogenetic activation of cells at specific locations in the virtual-reality context induced stable cellular representation of these locations. In characterizing neuronal excitability before and after this opto-plasticity, we found no significant change, consistent with a synaptic mechanism for the plasticity. We next developed optical methods to

probe synaptic transmission between presynaptic CA3 cells and postsynaptic CA1 cells, by simultaneous optogenetic stimulation of CA3 cells during optical recording of postsynaptic voltage responses in CA1 cells. We optically resolved both excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs) generated by optogenetic stimulation of CA3 neurons (n = 8 out of 22 recorded spiking cells showed EPSPs; n = 12 out of 22 recorded spiking cells showed IPSPs, N = 4 mice). These results establish methods for all-optical electrophysiology that can reveal synaptic and circuit-dynamics properties and plasticity in behaving mammals.

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Poster

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Program #/Poster #: P983.05

Topic: I.08. Methods to Modulate Neural Activity

Support: Stanford Graduate Fellowship

Title: Brainwide imaging and holographic control of transgenic zebrafish with red-shifted ChRmine channelrhodopsins created by structure-guided design

Authors: *T. BENSTER[1], A. S. ANDALMAN[2], Y. KIM[3], P. Y. WANG[3], M. INOUE[5], S. QUIRIN[4], C. RAMAKRISHNAN[3], R. CHEN[3], A. GERBINO[3], A. RAVENTOS[3], M. K. LO[3], K. E. KISHI[6], M. FUKUDA[6], E. F. X. BYRNE[3], E. THADHANI[3], J. M. PAGGI[3], H. E. KATO[6], S. DRUCKMANN[3], K. DEISSEROTH[7];
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Abstract: A long-sought goal in neuroscience is read/write access to an entire vertebrate brain at single-neuron resolution. In moving toward this aim, we solved the cryo-EM structure of the channelrhodopsin ChRmine and created three new transgenic zebrafish lines featuring novel ChRmine variants (designed based on the structure) with pan-neuronal expression during the larval stage when the whole brain is optically accessible. Using 3D two-photon imaging (512 pixels x 512 pixels x 10 planes) with 3D spatial light modulator (SLM) two-photon holographic stimulation (1536 pixels x 1536 pixels), we image tens of thousands of neurons at cellular resolution while stimulating up to 128 neurons across multiple brain regions, including the habenula and raphe nucleus. To guide and interpret collection of these datastreams, we

developed a new unsupervised denoising technique, based on Long Short-Term Memory networks (LSTMs), that automatically rejects stimulation artifacts as well as salt-and-pepper imaging noise. Together, this new approach permits single-trial analysis of brain-wide responses to concurrent optogenetic stimulation of specified cell-resolved ensembles. Across multiple fish (n=10), we observed that stimulating either the left or right habenula reliably allows measurement of the habenular projective field including distinct subpopulations of neurons in the medulla and raphe, and identification of putative interpeduncular nucleus (IPN) somata. Parametrically-titrated single-neuron stimulation in the habenular ensemble, from 2-32 neurons, revealed a ramping recruitment in the medulla commensurate with the number of neurons stimulated. For (n=2) fish, we explored the hypothesis that the raphe and habenula act in opposing fashion, as observed in prior work on behavioral state transitions. Using online analysis, we perform an initial demonstration of projective field tracing: we identify 32 neurons in the medulla/IPN that are recruited from optogenetic stimulation of the habenula, and use anatomy to identify 32 neurons in the raphe nucleus. We measure the projective field in response to these two stimulation groups across the entire medulla, as well as for a sham stimulation of 32 targets outside of the brain. A pixel is said to be contained in a projective field if the response exceeds a threshold that yields a 5% false discovery rate. By separately stimulating anatomically-identified raphe neurons or optogenetically-identified habenular efferents in the medulla, we find that these stimulations recruit largely orthogonal downstream cell populations, with a projective field overlap of only 7% despite the close proximity of recruited neurons.

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Poster

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Program #/Poster #: P983.06

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH K99 NS116122-02
AP Giannini Fellowship
Stanford Dean's Award Fellowship
NSF GRFP DGE-114747
NIH
HHMI

Title: Brainwide neural circuitry for controlling orofacial movements

Authors: *T. A. MACHADO[1], I. V. KAUVAR[1], Y. CHEN[1], J. KOCHALKA[1], S. BRADBURY[1], K. DEISSEROTH[2];
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Abstract: Even many simple movements are planned, initiated, and executed by the joint activity of neural circuitry distributed across the brain. But how does this widely-distributed circuitry influence downstream motor structures to control behavior? Mammalian orofacial motor nuclei in the brainstem represent an accessible target for comprehensive study of relevant circuitry in addressing this question. We first studied these behaviors in head-restrained mice using a new widefield optical imaging method, Cortical Observation by Synchronous Multifocal Optical Sampling (COSMOS) (Kauvar*, Machado*, et al., Neuron, 2020), to record with true simultaneity from over a thousand neuronal sources spread across the entirety of curved mouse dorsal neocortex at ~30 Hz; analysis suggests that individual COSMOS sources represent mixtures of signals from 1-15 neurons. Using a memory-guided lick-to-target task, we found that distributed neural activity throughout cortex encodes targeted licking actions, with no apparent spatial structure ($p < 0.05$, permutation test for spatial clustering of sources with similar tunings; 4 mice). We also found that across cortex, unaveraged (but not trial-averaged) activity correlations showed local structure (at distances < 1 mm, unaveraged correlations were consistently lower than trial-averaged correlations; $p = 0.0001$, paired t test; 4 mice). Finally, analysis of population dynamics revealed similar encodings of history-guided motor plans in unrelated cortical areas. We next asked how these distributed cortical representations are read out by the brainstem to drive movement. To address this question, we measured neural activity in brainstem circuits (that integrate descending inputs from cortex and elsewhere in the brain to drive orofacial movements) while similar movements were made in different behavioral contexts. During behavior in this paradigm, we simultaneously employed optogenetic inhibition in cortex, multiregion Neuropixels recordings from various brainstem nuclei, and 3D kinematic tracking of orofacial movements. These data were aligned to the Allen Atlas Common Coordinate Framework, where we co-registered information about neuronal connectivity (from rabies tracing and hydrogel-based brain clearing) and neuronal cell typology (using STARmap for in situ sequencing of the transcriptome in the brainstem). Combining these diverse data streams into a single coherent map may advance understanding of how distributed representations and descending commands drive specific movements--and of the essential differences between volitional and reflexive actions.

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Poster

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Topic: I.08. Methods to Modulate Neural Activity

Support: NIH BRAIN Initiative R01 EB028166
James S. McDonnell Foundation's Understanding Human Cognition Scholar Award
NSF FOUNDATIONS award NSF1926800

Title: Multi-region network models of brain-wide interactions underlying adaptive and maladaptive state transitions

Authors: *M. G. PERICH[1], T. BENSTER[2], A. S. ANDALMAN[3], C. SPENCER-SALMON[1], E. CARTER[1], K. DEISSEROTH[4], K. RAJAN[1];
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Abstract: During prolonged periods of stress, animals can switch from active to passive coping strategies to manage effort-expenditure. Such normally adaptive behavioral state transitions can become maladaptive in disorders such as depression. Here, we studied the neural mechanisms underlying behavioral state transitions using whole-brain neural recordings during maladaptive passivity induced by inescapable stress. Larval zebrafish expressing nuclear-localized GCaMP6s were exposed to a behavioral challenge protocol with repeated electric shocks. Initially, the fish showed vigorous tail movements, indicating an active coping (adaptive) strategy. The fish reduced movements as the shocks persisted, entering into a passive coping (maladaptive) state. We imaged whole-brain neural activity using two-photon microscopy and developed multi-region recurrent neural network (RNN) models to infer brain-wide directed interactions driving such maladaptive behavior by directly targeting the neural dynamics from upwards of 10,000 simultaneously-recorded neurons. Analysis of the trained RNN models revealed a specific change in directed interactions between two critical brain regions - the habenula and raphe nucleus - during the transition into passivity.
Using the directed interaction weights derived from the RNN models, we then performed Current-Based Decomposition (CURBD) to estimate the input currents from up to thirteen different brain regions driving each habenular neuron. We computed neural manifolds spanning these input currents across all habenular neurons to define subspaces within the habenular activity that independently captured the communication between other individual brain regions. At the onset of stress, there was an immediate and unique increase in currents from the raphe nucleus to the habenula. However, RNN models identified no similarly rapid change in the strength of directed interactions between these regions. As the animal lapsed into passivity, the strength of the raphe inputs to habenula decreased, with a concomitant change in the habenular interactions inferred from the RNN weights. This coincided with increases in currents to

habenula from other brain regions including the telencephalon and thalamus. This innovative combination of network modeling and neural dynamics analysis points to dual mechanisms with distinct timescales driving the behavioral state transition: early response to stress is mediated by reshaping the neural dynamics within a preserved network architecture, while long-term state changes correspond to altered directed interactions between neural ensembles in distinct brain regions.

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Poster

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Program #/Poster #: P983.08

Topic: I.08. Methods to Modulate Neural Activity

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Title: Cardiogenic control of affective behavioral state

Authors: B. HSUEH[1], ***R. CHEN**[1], S. RANDLES[1], D. D. TANG[1], C. RAMAKRISHNAN[1], Y. JO[1], M. RAFFIEE[1], Y. KIM[1], M. INOUE[1], S. KIM[1], L. TAN[1], D. KIM[1], A. CROW[1], C. RAJA[1], K. E. EVANS[1], M. GOUBRAN[2], K. DEISSEROTH[3];
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Abstract: Physiological theories of emotion, proposed over a century ago, hypothesized that (rather than a unidirectional brain to body flow of information) the reverse direction could be important and even dominant (for example, that rapid heartbeats alone might induce fear responses and serve not merely as a consequence of central emotional processing). This important hypothesis had not been formally and precisely testable until now, since to test (for example) how altered cardiac rhythms might affect emotional or affective state, nonspecific interventions such as broadly-active stimulants or painful shocks would be required. In fact, even with optogenetics this would not have been possible until recently, since existing opsins were not sensitive enough to control a large organ like the heart with enough precision and power. To

address this question, we developed a noninvasive optogenetic pacemaker utilizing 1) ChRmine under control of the mouse cardiac troponin T promoter delivered via AAV9 for enhanced tropism in cardiac tissue (AAV9-mTNT::ChRmine-2A-oScarlet) and 2) wearable micro-LED optics, which enabled precise control of cardiac rhythms up to 1000 beats per minute in freely-moving mice within a safe range of illumination power similar to conventional optogenetics. We tested the behavioral impact of truly precise and specific induced cardiac changes on behavior and affective state. We found that this primary and direct tachyarrhythmia indeed potently enhanced anxiety-like behavior, demonstrating causal impact of the body to brain axis (n=16). This effect could only be observed in risky contexts, appreciable by the brain, revealing that both directions (brain to body and body to brain) are synergistically required to understand behavior (n=16). Then, using a whole brain activity screen in in double transgenic TRAP2:Ai14 mice, we mapped regions of the brain that were activated by these imposed cardiac rhythms, and identified posterior insular cortex as a potential mediator of bottom-up cardiac signal processing (n=8). We then showed that simultaneous optogenetic inhibition of posterior insular cortex with iC++ during optical cardiac pacing was sufficient to reverse the induced behaviors, implicating this region as a necessary site for mediating cardiogenic anxiety-like and apprehensive behavior (n=8). Our findings offer insights into the specific mechanisms by which such bodily signals can causally influence complex behaviors, reveal that body and brain must be considered together; we also present robust and generalizable tools for non-invasive, temporally precise control of precision-targeted cells, tissues, and organs throughout the body.

Disclosures: B. Hsueh: None. **R. Chen:** None. **S. Randles:** None. **D.D. Tang:** None. **C. Ramakrishnan:** None. **Y. Jo:** None. **M. Raffiee:** None. **Y. Kim:** None. **M. Inoue:** None. **S. Kim:** None. **L. Tan:** None. **D. Kim:** None. **A. Crow:** None. **C. Raja:** None. **K.E. Evans:** None. **M. Goubran:** None. **K. Deisseroth:** None.

Poster

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Stanford University Walter V. and Idun Berry Postdoctoral Fellowship
NIH K99/R00 Pathway to Independence Award (1K99NS119784)
Stanford Bio-X Undergraduate Summer Research Program
Gatsby Foundation Grant

Title: Mapping Postnatal Dynamics of Single-cell 3D Genome Architecture in the Developing Mouse and Human Cerebellum

Authors: *L. TAN[1], R. CHEN[1], J. SHI[1], I. COBOS[2], L. DUNCAN[3], K. DEISSEROTH[1,3,4]; [1]Bioengineering, [2]Pathology, [3]Psychiatry and Behavioral Sci., [4]Howard Hughes Med. Inst., Stanford Univ., Stanford, CA

Abstract: Throughout neurodevelopment, spatiotemporal expression patterns of thousands of genes are precisely orchestrated, in ways that presumably help guide the proper formation of massive interconnected neural and glial networks. How does such complex regulation of gene expression occur at the cellular level? One emerging mechanism involves three-dimensional (3D) genome architecture. Recently, alteration in 3D genome architecture has been implicated in multiple neurodevelopmental disorders, including autism spectrum disorder (ASD), schizophrenia, and intellectual disability. Despite its importance, our understanding of 3D genome architecture in the developing brain is severely limited by the lack of high-resolution in vivo technology for comprehensive single-cell datastreams. To bridge this longstanding technology and knowledge gap, we previously developed a high-resolution single-cell chromatin conformation capture (3C/Hi-C) method—Dip-C—and resolved the first 3D genome structure of a single human cell with 20 kb (~100 nm) resolution. Recently, we applied Dip-C to 3,517 cells from the developing mouse cortex and hippocampus. We found 3D genome "structure types" that underlie diverse transcriptional cell types in the brain. Surprisingly, we discovered a multi-scale 3D genome transformation in 3 main cell lineages (neurons, astrocytes, and oligodendrocytes) in the first month of life. For example, in neurons, many regions across the genome—including the majority of chromosomes 7, 17, and 19—move inward.
Here we turn to the cerebellum, which harbors the majority of neurons in the mammalian brain and which undergoes unusually late development. For example, generation of granule cells occurs almost entirely after birth in mice and persists until the first postnatal year in humans. The cerebellum is also the most consistently affected brain region in ASD. However, little is known about the dynamics of 3D genome architecture in the developing cerebellum. We created the first single-cell 3D genome atlas of 3,797 cells from developing mouse and human cerebellum of both sexes. Unexpectedly, 3D genome changes that we previously observed in the mouse cortex and hippocampus (as well as in olfactory sensory neurons) were mostly absent from the cerebellum, suggesting a fundamental dichotomy in 3D genome regulation between forebrain and hindbrain. Instead, 3D genome of cerebellar granule cells undergoes highly cell type-specific rewiring throughout life (0-2 years in mice). Our findings provide new molecular insight into the cerebellum's unique mode of development, and may point to critical processes relevant to altered development as in ASD.

Disclosures: L. Tan: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); L.T. is an inventor on a patent application US16/615,872 filed by Harvard University that covers Dip-C.. **R. Chen:** None. **J. Shi:** None. **I. Cobos:** None. **L. Duncan:** None. **K. Deisseroth:** None.

Poster

P006. Cell Migration

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Program #/Poster #: P006.01

Topic: A.01. Neurogenesis and Gliogenesis

Support: RO1 AA027111

Title: Microglia Distribution in Early Postnatal Cerebellum

Authors: E. C. PLUNK[1], M. B. STOESSEL[2], M. Y. CEALIE[2], P. D. DREW[4], K. D. PHELAN[5], *A. K. MAJEWSKA[3];
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Abstract: Microglia are traditionally known to be the resident immune cells of the brain; however, recent research has shown that they serve several other functions, including synaptic plasticity, phagocytosis of apoptotic cells, and phagocytic removal of debris. Microglia also have brain region specific morphologies, transcriptome profiles, and roles, and cerebellar microglia in particular seem to be distinct from microglia in other brain areas. Cerebellar microglia are less dense, have fewer processes, show different dynamic movements and have a different transcriptomic profile compared to those in other regions of the brain. Developmentally, cerebellar microglia are more phagocytic than those in other brain areas, clearing Purkinje cells that undergo developmental apoptosis. Microglial phenotype may be altered by these developmental events as well as the cerebellum's distinct structure and cell types, and its late development in comparison to the rest of the brain. To further characterize the development of cerebellar microglia, we analyzed the migration of microglia from the white matter to the grey matter of the mouse cerebellum by examining multiple developmental time points starting at post-natal day (P) 3.5 in Cx3Cr1 G/- mice, which fluorescently label all microglia in the brain. We investigated microglial density and distribution in white and grey matter at each time point and found that microglial migration into cerebellar gray matter occurs late in development after P10. At this time point, Purkinje cells are starting to form a monolayer and extend dendritic processes, suggesting that this early development does not require direct microglial contact. Understanding the behaviors of developing cerebellar microglia could give insight as to why they differ from microglia in other brain regions, as well as shedding light on their role in neurodevelopmental and neurodegenerative diseases involving the cerebellum.

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Poster

P006. Cell Migration

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Program #/Poster #: P006.02

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH R01NS104026

Title: Cas Adaptor Proteins Regulate Cortical Migration and Lamination

Authors: *W. WONG[1], B. ZAREIAN[2], Y. C. WONG[1], E. ZAGHA[2], M. M. RICCOMAGNO[1]; [1]Molecular, Cell, & Systems Biol., [2]Psychology, Univ. of California, Riverside, Riverside, CA

Abstract: Laminar organization of the cortex requires neurons to exit the cell cycle, locomote, and assemble into six distinct layers. During migration, neurons encounter multiple permissive and repulsive cues, yet how these guidance cues are integrated within migrating cells is not well understood. To uncover the signaling events essential for radial migration, we studied how the three embryonically expressed Cas signaling adaptor proteins (p130Cas, CasL, and Sin) function during cortical development. The Cas family of proteins have been hypothesized to mediate integrin and guidance cue signal transduction during neural development. Here, we provide *in vivo* genetic evidence that Cas proteins serve a functional and redundant role during cortical lamination and axon guidance. Cas triple conditional knockout (*CasTcKO*) mice display severe cortical phenotypes that resemble cobblestone lissencephaly. In *CasTcKO* brains, ectopic clusters of neurons form beyond the marginal zone and sometimes invade the subarachnoid space. These ectopias are innervated by L1-CAM+ axon bundles. Furthermore, both deep and superficial layer neurons are mislocalized in *CasTcKO* mutants. Defects in neuronal positioning appear to be non-neuronal autonomous and may be due to a breakage in the basal lamina. Electrophysiological and behavioral studies revealed distinct functional deficits in *CasTcKO* mice, providing insights into the consequences of altered lamination on cortical processing. Overall, our data support an essential role for Cas adaptor proteins during the formation of cortical circuits.

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Poster

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Support: Knowledge fellowship
Arsenov foundation

Title: Embryonic retinopetal projections in human

Authors: *I. BYSTRON[1], C. BLAKEMORE[2];
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Abstract: It is universally accepted that all neurons of the retina, in all mammalian species, are produced from progenitor cells in the retinal neuroepithelium, and that locally-generated ganglion cells are the first neurons to appear in the embryonic eye. Here we provide the evidence that the human eye, at the earliest stages of its development, contains a unique neuronal population **precocious retinopetal neurons (PRN)** that is generated in the brain.
We have developed a new approach to reconstructing cells in sections of the embryonic human brain and retina by rapid, high-resolution volume rendering of multichannel 3D confocal data-sets to reveal neurogenesis in the embryonic eye and the possible contribution to retinal development of neurons born elsewhere. Early embryonic tissue was obtained from the Human Developmental Biology Resource UK. Here we provide preliminary evidence that neuronal development in the human retina starts with precocious neurons invading the retina from ventral diencephalon. These cells, might be important in the cascade of developmental events leading to the formation of the human retina and the optic nerve. Long processes of the first neurons in the retina are not conventional nerve fibres. Although they do not express MAP2, GAP43, Tag1- the markers which we found in the first ganglion cells at E40-43 - it is possible that PRN neurons signal chemically to the processes of stem cells in neural and pigmental retina, with which they make intimate contacts. Moreover we also found bipolar TU-20-positive neurons scattering through the periocular mesenchyma. Some of them extended TU20-positive processes into the neural retina. We applied a battery of molecular markers to reveal that these periocular neurons do not belong to the diverse neural crest cell population delaminating from the closing neural tube and migrating through the cephalic mesenchyme. Invasion of the developing retina by extraretinal neurons from the diencephalon and by processes of neurons in the mesenchyme has not been described in any other mammalian species. The genetic and molecular mechanisms that underlie the relationship between PRN, periocular neurons and retinal stem cells remain to be elucidated.

Disclosures: I. Bystron: None. **C. Blakemore:** None.

Poster

P006. Cell Migration

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P006.04

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH 5P01NS083513

Title: Postnatal streams of young migrating interneurons contribute to late temporal lobe development in the infant human brain

Authors: *S. BIAGIOTTI[1], M. A. NASCIMENTO[2], V. HERRANZ-PÉREZ[5], S. GONZALEZ[5], J. GARCIA-VERDUGO[5], E. J. HUANG[3], A. ALVAREZ-BUYLLA[4], S. F. SORRELLS[1];
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Abstract: In the infant human brain, young neurons continue to migrate in the subventricular zone along the walls of the lateral ventricles into the rostral migratory stream and the olfactory bulb. It has recently been demonstrated that this postnatal migration also includes multiple regions of the forebrain through a coalesced medial migratory stream and distributed arc of migratory cells into the cingulate and superior frontal gyrus. Continued postnatal recruitment of migrating neurons into other parts of the infant human brain has not been investigated. In this study we examined the temporal lobe from ages 18 gestational weeks through 2 years of age to look for migrating neurons expressing doublecortin (DCX) and polysialylated neural cell adhesion molecule (PSA-NCAM). We find that the human temporal lobe lateral ventricles are surrounded by a distributed arc of migratory neurons similar to what is observed in the forebrain as well as individual young neurons with an elongated nucleus and a leading process indicative of migration. These neurons are DLX2+ and TBR1-, suggesting that this is a late migration of interneurons into the temporal lobe. Following this, we sought to determine the subtype of interneuron these young cells were becoming. We found that this late migration correlates with an increase in Calretinin+ cells. Our study reveals a late contribution of young migrating interneurons into the temporal lobe that aid in the development of the infant human brain. This late migration of young neurons throughout the human infant brain may indicate critical times when circuits are plastic, as well as sensitive periods when environmental disturbances could impact brain development.

Disclosures: S. Biagiotti: None. **M.A. Nascimento:** None. **V. Herranz-Pérez:** None. **S. Gonzalez:** None. **J. Garcia-Verdugo:** None. **E.J. Huang:** None. **A. Alvarez-Buylla:** F. Consulting Fees (e.g., advisory boards); Neurona Therapeutics. **S.F. Sorrells:** None.

Poster

P006. Cell Migration

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Program #/Poster #: P006.05

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH R15NS108183

Title: Characterization of neuroblast migration along key cellular substrates in the developing porcine brain

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Abstract: Neurogenesis, the birth of new neurons, and neuronal migration underlies the development of the human cerebral cortex. In postnatal and adult mammalian brains, immature neurons called "neuroblasts" generated in the subventricular zone (SVZ), undergo protracted migration (millimeters to centimeters, depending on the species) to reach their final destinations in the expanding neocortex and contribute to brain development/plasticity. Recent analyses of postmortem tissue revealed that humans possess robust migratory pathways of late arriving interneurons oriented towards the frontal lobe, namely the prefrontal cortex. However, the cellular mechanisms enabling these unique migratory routes remain unclear. Currently, our understanding of this process in human brain development is fragmentary, largely due to the diverging developmental features of well-studied rodent models during postnatal development. Here we use the neonatal piglet (*Sus scrofa domesticus*) to study the occurrence, anatomical distribution, and expression profile of SVZ-derived neuroblasts in a larger, gyrencephalic brain that more closely resembles its human counterpart. Tissue at postnatal day 0 (p0), p16, and p42 was used to evaluate the number of neuroblasts associated with cellular substrates in key regions of the SVZ of newborn piglets. Using a combined approach of multiplex labeling and microscopy, our current results demonstrate that the porcine SVZ contains a large, heterogeneous population of DCX^+ neuroblasts that are maintained throughout early postnatal development at all ages. Immunohistochemical analyses revealed DCX^+ clusters co-express the neuronal cell migration marker polysialylated neural cell adhesion molecule (PSA-NCAM) as well as the GABAergic interneuron marker ($GABA^+$) and regionally specific transcription factors ($SCGN^+SP8^+$) from the caudal- (CGE) and lateral ganglionic eminence (LGE) progenitor domains in the ventral forebrain. Moreover, we found that DCX^+ neuroblasts are encased by astrocytic ($GFAP^+$) processes and tightly associated with blood vessels ($IB4^+$) in the SVZ. Taken together, these data suggest that the vast majority of the SVZ-derived DCX^+ clusters are GABAergic interneurons that originate in the subpallial region of the telencephalon and migrate tangentially utilizing vascular scaffolds to the neocortex, which is likely to contribute to cortical plasticity in the postnatal piglet. Findings from this study will better inform future studies evaluating human cortical development and neuronal migration in normal and diseased states.

Disclosures: D.D.L. Porter: None. **S.N. Henry:** None. **S. Ahmed:** None. **P.D. Morton:** None.

Poster

P006. Cell Migration

Location: Virtual Only

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Program #/Poster #: P006.06

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH Grant NS108367

Title: Suppression of WNT5A-mediated chemoattraction by atypical cadherin CELSR1 prevents inappropriate migration of facial branchiomotor neurons

Authors: *D. HUMMEL[1], A. BECKS[1], H. MEN[2], E. BRYDA[3], D. GLASCO[4], A. CHANDRASEKHAR[1];
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Abstract: As the central nervous system develops, differentiating neurons often migrate extensively prior to forming the circuits vital to cognitive and motor function. Within the vertebrate hindbrain, Facial Branchiomotor (FBM) neurons migrate caudally from rhombomere 4 (r4) to r6 and assemble into the circuits that drive facial and jaw movements. While several components of the Wnt/PCP (Planar Cell Polarity) pathway have been investigated based on their required role in initiating FBM neuron migration, much less is known regarding the mechanisms regulating directionality. Our lab discovered that in mice lacking the Wnt/PCP component CELSR1, many FBM neurons inappropriately migrate rostrally into r3. Tissue-specific knockouts of *Celsr1* indicate that *Celsr1* acts non-cell autonomously in the ventricular zone rostral to r4 to block rostral migration of FBM neurons. Intriguingly, *Celsr1* and *Wnt5a* are expressed in overlapping domains within the ventricular zone rostral to r4. Based on these findings, we hypothesize that *Celsr1* suppresses WNT5A activity rostral to r4 to block inappropriate rostral migration of FBM neurons. We have now successfully tested three predictions of this model.
If WNT5A functions as a chemoattractant allowing for rostral migration in *Celsr1* mutants, then rostral migration should be suppressed in *Celsr1; Wnt5a* double knockouts. Indeed, FBM neurons in *Celsr1; Wnt5a* double knockout embryos never migrate rostrally and undergo normal caudal migration. Next, if WNT5A is the chemoattractant inducing rostral migration of FBM neurons in *Celsr1* knockouts, then rostral migration should be induced in wildtype hindbrains with WNT5A-coated beads placed in r3. We found that FBM neurons migrate rostrally toward WNT5A beads placed in r3 and r2 in >80% of wildtype explants, suggesting that excess WNT5A can overcome endogenous *Celsr1*-mediated suppression. Importantly, rostral migration toward the beads is significantly reduced in *Dvl2* mutants, demonstrating that migration is dependent on WNT5A-DVL2 signaling. Finally, our model predicts that rostral migration of FBM neurons should be greatly enhanced in *Celsr1* mutants overexpressing *Wnt5a* in r3. As expected, when a *Wnt5a* gain-of-function allele is used to overexpress *Wnt5a* in r3 of *Celsr1* mutants, the population of rostrally migrating FBM neurons is substantially larger than in control mutants, further supporting the chemoattractant model. These results reveal a novel role for a Wnt/PCP component in regulating neuronal migration by suppressing chemoattraction.

Disclosures: D. Hummel: None. **A. Becks:** None. **H. Men:** None. **E. Bryda:** None. **D. Glasco:** None. **A. Chandrasekhar:** None.

Poster

P006. Cell Migration

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Program #/Poster #: P006.07

Topic: C.10. Brain Injury and Trauma

Support: National Institutes of Health [R01-NS117757 (D.K.C.)]
National Science Foundation [DGE-1845298 (E.M.P.)]
Department of Veterans Affairs [RR&D Career Development Award IK2-RX003376 (J.C.O.D.)]
Center for Undergraduate Research and Fellowships at the University of Pennsylvania [K.V.T.H.]

Title: Development and characterization of an implantable tissue-engineered rostral migratory stream for directed neuronal replacement

Authors: *E. M. PURVIS[1,2,3], J. C. O'DONNELL[1,2], K. V. T. HELM[1,2], D. O. ADEWOLE[1,2,4], Q. ZHANG[5], A. D. LE[5,6], D. K. CULLEN[1,2,4];
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Abstract: Brain injury and neurodegenerative disease can result in long-term neuronal loss that is exacerbated by the limited regenerative capacity of the central nervous system. The rostral migratory stream (RMS) facilitates neuroblast migration from the subventricular zone to the olfactory bulb throughout adulthood. Brain lesions attract neuroblast migration out of the RMS, but resultant regeneration is insufficient without experimental intervention. Our lab has biofabricated a “living scaffold” that is implanted to enhance and redirect endogenous neuroblast migration from the subventricular zone to neuron-deficient brain regions. This approach utilizes the first implantable, biomimetic tissue-engineered RMS (TE-RMS), designed to leverage the brain’s natural mechanism for sustained neuronal replacement by replicating the native RMS in order to direct neuroblasts to distal sites of injury. Our previous work has characterized the structure of the TE-RMS fabricated from primary rat postnatal cortical astrocytes (rat TE-RMS). We have recently demonstrated that astrocyte-like cells can be non-genetically derived from adult human gingiva mesenchymal stem cells (n = 3 de-identified donors) and used for TE-RMS fabrication (human TE-RMS), and that key proteins (ezrin, robo2, and glial fibrillary acidic

protein) enriched in the endogenous adult male rat RMS (n = 5) are also enriched in the rat (n = 9) and human (n = 5) TE-RMS, but not in planar astrocyte sister cultures (n = 6 for rat and human). Additionally, we demonstrate that astrocytes in the endogenous RMS possess an elongated nuclear shape and distinct cytoskeletal arrangement compared to surrounding protoplasmic astrocytes, and that astrocytes in the rat TE-RMS mimic this distinct astrocytic nuclear shape and intermediate filament arrangement whereas planar astrocyte sister cultures do not. Furthermore, the human TE-RMS facilitates directed migration of immature rat cortical neurons *in vitro* (n = 4) at an average migration rate of at least 56 microns/hour which is comparable to neuroblast migration through the endogenous RMS, whereas acellular collagen (n = 4) and acellular collagen and laminin (n = 5) control columns do not direct immature neuronal migration *in vitro*. Finally, human TE-RMSs implanted in athymic rat brains redirect migration of neuroblasts out of the endogenous RMS (n = 6). The rat and human TE-RMS mimic the basic structure and function of the endogenous rat RMS. By emulating the brain's most efficient means for directing neuroblast migration, the TE-RMS offers a promising new regenerative medicine therapy for endogenous neuronal replacement following brain injury.

Disclosures: E.M. Purvis: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); U.S. Provisional Patent App. 63/197,007 titled "Tissue-engineered rostral migratory stream for neuronal replacement". **J.C. O'Donnell:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); U.S. Provisional Patent App. 63/197,007 titled "Tissue-engineered rostral migratory stream for neuronal replacement". **K.V.T. Helm:** None. **D.O. Adewole:** None. **Q. Zhang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); U.S. Provisional Patent App. 63/197,007 titled "Tissue-engineered rostral migratory stream for neuronal replacement". **A.D. Le:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); U.S. Provisional Patent App. 63/197,007 titled "Tissue-engineered rostral migratory stream for neuronal replacement". **D.K. Cullen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); D.K.C. is a co-founder of two University of Pennsylvania spin-out companies concentrating in applications of neuroregenerative medicine: INNERVACE, Inc. and Axonova Medical, LLC., U.S. Patent App. 15/534,934 titled "Methods of promoting nervous system regeneration", U.S. Provisional Patent App. 63/197,007 titled "Tissue-engineered rostral migratory stream for neuronal replacement".

Poster

P063. Developmental Disorders: Molecular and Cellular Mechanisms

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P063.01

Topic: A.07. Developmental Disorders

Support: NIH Grant NS105804
CURE Epilepsy
Dravet Syndrome Foundation
Brain Research Foundation

Title: Intrinsic and synaptic mechanisms alter thalamic neuron function in a Dravet syndrome mouse model

Authors: *C. STUDTMANN[1,2], M. TOPOLSKI[1], M. SAFARI[2,1], S. A. SWANGER[1,3,4];
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Abstract: Dravet Syndrome (DS) is an infantile epileptic encephalopathy caused by mutations in the voltage-gated sodium channel $Na_V1.1$, which leads to hyperexcitable brain activity during development. This altered excitability causes persistent dysfunction across brain circuits, resulting in a broad phenotypic profile including non-convulsive (absence) seizures, attention deficits, and sleep disruption. Corticothalamic (CT) circuits are responsible for diverse informational processing and play a particularly important role in regulating attention and sleep. Disrupted CT circuit activity contributes to a variety of phenotypes in DS models and thus offers a promising therapeutic target. However, the precise cellular and synaptic mechanisms underlying CT circuit dysfunction remain elusive. Here, we sought to identify the cellular and synaptic mechanisms underlying pathological somatosensory thalamic circuit activity in a DS mouse model with $Na_V1.1$ haploinsufficiency, thereby revealing therapeutic targets to correct circuit function. Electrophysiological results revealed synaptic-level alterations in the somatosensory thalamus, including an input-specific reduction in glutamatergic synaptic input to the ventral posterolateral (VPL) thalamic nucleus and a significant increase in GABAergic input to the same region. Interestingly, synaptic input to the adjacent ventral posteromedial (VPM) thalamic nucleus remained unaltered. Glutamatergic synaptic input to the reticular nucleus of the thalamus (nRT), an inhibitory neuron population providing the primary inhibitory input to the VPL and VPM, exhibited an input-specific reduction without a change in overall glutamatergic input. Furthermore, all three neuron populations exhibited distinct alterations to intrinsic excitability in the disease model. VPL neurons exhibited hypoexcitability in response to depolarization, while VPM neurons were hyperexcitable in DS mice. We also revealed two nRT neuron populations with altered responses to depolarization including a low tonically firing population and a burst firing population. These changes in depolarization-induced firing were accompanied by alterations in resting membrane potential and hyperpolarization-induced rebound burst firing in both nRT and VPL neurons. These data indicate that diverse synapse- and cell-type-specific mechanisms in multiple neuron populations may contribute to somatosensory thalamic dysfunction in this DS model, thus providing new opportunities for therapeutic intervention.

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Poster

P063. Developmental Disorders: Molecular and Cellular Mechanisms

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P063.02

Topic: A.07. Developmental Disorders

Title: Characterization of the Okur Chung Neurodevelopmental Syndrome associated CK2K198RMutation

Authors: *D. M. CAEFER[1], N. Q. PHAN[1], J. P. O'SHEA[1], J. C. LIDDLE[2], J. L. BLASBAUGH[2], A. V. TZINGOUNIS[1], D. SCHWARTZ[1];
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Abstract: Okur-Chung Neurodevelopmental Syndrome (OCNDS) was first described in 2016 and since then over 120 additional cases have been reported. The syndrome is characterized by delayed psychomotor development, intellectual disability, behavior abnormalities, and variable dysmorphic facial features. Genetically, OCNDS is associated with heterozygous mutations to the CSNK2A1 gene, which encodes the alpha subunit of casein kinase II (CK2). Numerous OCNDS associated mutations have been identified, with the most frequent being lysine-198 to arginine (K198R). Lysine-198 is located in the activation segment of the kinase, a well-conserved region important for kinase activity and substrate recognition. Despite the expanded number of identified mutations since the first characterization of OCNDS, little is known about the underlying molecular mechanisms, beyond speculation of altered CK2 activity. As a first step towards understanding how the CK2K198R mutation ultimately results in a disease phenotype, we generated high resolution specificity motifs of CK2WT and CK2K198R using the Proteomic Peptide Library approach (ProPeL). These experiments revealed that contrary to prior speculation the OCNDS associated CK2K198R mutation does not result in a loss of function, but rather shifts the substrate specificity of CK2. Broadly speaking the mutation leads to a decreased preference for acidic residues in the +1 position and an altered phosphoacceptor preference. To elucidate how the CK2K198R mutation contributes to the OCNDS disease phenotype we developed a probabilistic strategy to identify phosphorylation sites that could be differentially phosphorylated by CK2K198R relative to CK2WT. Due to the neurodevelopmental nature of OCNDS, and the known enrichment of CK2 in the axonal initial segment, we focused our predictions of altered phosphorylation sites to ion channels localized to the axon. This search identified several known CK2 targets, including KCNQ2. To test our predicted shifts in phosphorylation, HEK293T cells were transfected with KCNQ2 and CK2WT, CK2K198R, or

CK2KD, lysed, enriched for KCNQ2 by immunoprecipitation, and analyzed by LC-MS/MS. Preliminary analyses of immunoprecipitated KCNQ2 indeed suggest differing phosphorylation states dependent on co-expression with either CK2WT or CK2K198R. Future experiments will expand on these results to further investigate the effect of CK2K198R on KCNQ2 phosphorylation and additional CK2 targets with the aim of revealing the molecular mechanisms underlying OCNDS.

Disclosures: D.M. Caefer: None. **N.Q. Phan:** None. **J.P. O'shea:** None. **J.C. Liddle:** None. **J.L. Blasbaugh:** None. **A.V. Tzingounis:** None. **D. Schwartz:** None.

Poster

P063. Developmental Disorders: Molecular and Cellular Mechanisms

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Program #/Poster #: P063.03

Topic: A.07. Developmental Disorders

Support: EY029166
NS103172
MH107367
AI132122
HG004659
HG009889
5 F32 NS112654-03

Title: Mechanistic and phenotypic convergence of congenital dm1 and rett syndrome

Authors: *K. MORELLI, W. JIN, S. SHATHE, G. W. YEO;
UCSD, La Jolla, CA

Abstract: Myotonic dystrophy type 1 (DM1) is a multisystem disorder caused by a CTG microsatellite repeat expansion (MRE) in the *DMPK* gene. While DM1 is the most common adult-onset muscular dystrophy, CTG expansions greater than 1500 bases result in congenital forms (cDM1) that manifest as neurodevelopmental defects. However, the molecular mechanism for how CTG MREs affect human cortical development is unknown. We detected CUG foci early during differentiation of three-dimensional cortical organoids from cDM1 patient-derived induced pluripotent stem cells. Toxic RNA expression causes disruption of widespread protein-RNA substrate interactions due to hyper-phosphorylation of RNA binding protein CELF2, which we reverse by CRISPR/Cas13d-mediated elimination of expanded CUG RNA. Enhanced CLIP analysis reveals impaired RNA metabolism of genes that are critical for excitatory synaptic signaling, including genes that are principal components of the MECP2 pathway responsible for Rett Syndrome (RTT). Strikingly, we uncover that cDM1 and RTT cortical organoids have

convergent phenotypes such as glutamate toxicity and neuronal loss, preventable by a NMDA antagonist. Our findings explain the molecular etiology of, and provide treatment avenues for, cDM1.

Disclosures: K. Morelli: None. **W. Jin:** None. **S. Shathe:** None. **G.W. Yeo:** None.

Poster

P063. Developmental Disorders: Molecular and Cellular Mechanisms

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Program #/Poster #: P063.04

Topic: A.07. Developmental Disorders

Support: NIH/NIAAA R01AA026272
NIH/NIAAA R01AA025215
Scott Gentle Foundation

Title: Pharmacological blockage of Kcnn2 improves the impaired cognitive flexibility in the mouse model of FASD

Authors: *S. MOHAMMAD, L. WANG, M. TORII, K. HASHIMOTO-TORII;
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Abstract: Both genetic and environmental factors affect the embryonic brain development. Alcohol exposure is one of such environmental factors, and thus increases the risk of intellectual disability and other neuropsychiatric conditions. Our recent studies revealed that molecular responses against alcohol exposure happen in a stochastic and heterogeneous manner among neural progenitor cells. Those prenatal molecular events are associated with the changes of the gene expression in adulthood. Among those changes of the gene expressions, the expression of Potassium Calcium-Activated Channel Subfamily N Member 2 (Kcnn2) is highly increased in the pyramidal neurons in the motor cortex of prenatally alcohol-exposed (PAE) mice. Furthermore, postnatal blockage of Kcnn2 improves motor skill learning deficits in the PAE mice. Similarly, the increased expression of Kcnn2 was found in the prefrontal cortex (PFC) in PAE mice that show reversal learning deficits in the water T-maze. Therefore, we administered a Kcnn2 blocker, Lei-Dab7, intranasally starting at postnatal day 40 to the PAE mice. 10 minutes post intranasal application of Lei-Dab7, the binding to the neurons that express Kcnn2 was confirmed in the PFC as well as other brain regions. Daily administration of Lei-Dab7 starting one day before reversing the platform in the water T-maze improved reversal learning deficits in the PAE mice. Altogether, these results suggest Kcnn2 blocker as a new pharmacological intervention for cognitive inflexibility and other learning disabilities in the Fetal Alcohol Spectrum Disorders (FASD).

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Poster

P063. Developmental Disorders: Molecular and Cellular Mechanisms

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P063.06

Topic: A.07. Developmental Disorders

Title: Electrophysiological Properties of Neurons in Cortical Organoids Derived from Rett Syndrome and CDKL5 Deficiency Patients

Authors: *W. WU[1], P. NEGRAES[5], H. YAO[2], J. WANG[1], C. A. TRUJILLO[6], A. R. MUOTRI[3], G. G. HADDAD[4];
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Abstract: CDKL5 deficiency disorder (CDD) and Rett syndrome (RTT) are two severe forms of X-linked Autism Spectrum Disorder with largely overlapping clinical symptoms. Epileptic seizures during early childhood are common, suggesting common alterations in neuronal function between CDD and RTT. Recently, we generated cortical organoids derived from CDD and RTT patients, as well as control individuals (i.e., healthy parental subjects). To investigate whether/how electrophysiological properties are altered, we performed whole-cell patch-clamp recordings in neurons of CDD and RTT organoids. We found that the # of action potentials (APs) (evoked from -70 mV to 200 ms-long injections of 50 pA current) in CDD neurons (1.90±0.15, n = 40) and RTT neurons (1.43±0.15, n = 23) were significantly higher than those in CDD control (1.07±0.08, n = 35) and RTT control (1.12±0.09, n = 33) ($p<0.05$, 1-way ANOVA). Further, the minimum currents (rheobase) injected to evoke an AP in CDD neurons (12.0±1.1 pA, n = 21) and RTT neurons (8.75±1.48 pA, n = 12) were significantly lower than those in CDD control (18.9±1.3 pA, n = 20) and RTT control (16.1±1.73 pA, n = 16), ($p<0.05$, 1-way ANOVA), suggesting increased neuronal excitability in both CDD and RTT organoids. Since voltage-gated ion channels are required for AP firings, we further used voltage-clamp techniques to dissect the densities of Na^+ and K^+ currents (I_{Na}, I_K). The peak I_{Na} densities (at -20mV) from CDD organoids and RTT organoids were 143.5±36.1% and 87.5±17.9% higher than those in corresponding controls ($p<0.01$, n=32-40). Additionally, the peak I_K densities (at +80mV) from CDD organoids and RTT organoids were 59.9±13.6% and 73.2±15.5% higher than those in corresponding controls ($p<0.01$, n=32-40), indicating increased channel activities in early CDD and RTT neurons. Together, our study revealed intrinsic neuronal hyperexcitability and upregulated function of voltage-gated Na^+ and K^+ channels in both CDD and RTT

organoids, which may contribute to the cellular electrophysiologic basis for their common symptoms such as early epilepsy.

Disclosures: W. Wu: None. **P. Negraes:** None. **H. Yao:** None. **J. Wang:** None. **C.A. Trujillo:** None. **A.R. Muotri:** None. **G.G. Haddad:** None.

Poster

P063. Developmental Disorders: Molecular and Cellular Mechanisms

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Program #/Poster #: P063.07

Topic: A.07. Developmental Disorders

Support: MDBR-18-106-CDKL5

Title: Epigenetic and transcriptomic post-mortem analyses in BA17 cortex of CDKL5-deficiency disorder patients

Authors: R. PIZZO, D. COMAI, A. LAURIA, S. DEVI, F. ANSELMI, A. GURGONE, S. OLIVIERO, *M. GIUSTETTO;
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Abstract: Cyclin-dependent kinase-like 5 (CDKL5) deficiency disorder (CDD) is a rare neurodevelopmental disease (NDD) caused by mutations in the CDKL5 gene, located on the X chromosome. The clinical features of CDD patients span from intellectual disability to poor language skills, impaired motor development, autistic-like tendencies, and severe early onset epileptic seizures. Moreover, CDD patients suffer from sensory defects, such as auditory and cortical visual impairments. CDKL5, a serine/threonine kinase, can shuttle between the nuclear and the cytoplasmic compartments. In the cytoplasm, CDKL5 regulates the formation and the functional maturation of dendritic spines as well as controlling cellular cytoskeleton dynamics. On the other hand, it has been suggested that CDKL5 interacts with epigenetic factors, i.e.: MeCP2, DNMT1 and HDAC4, raising the hypothesis that CDKL5 might regulate gene expression. However, the impact of CDKL5 mutation in the nucleus remains largely obscure even though mounting evidence place chromatin regulation, along with synaptic function, as the most affected molecular pathways of NDD-causative gene mutations. In the present study, by exploiting post-mortem BA17 cortex samples from two CDD individuals and two neurotypical age- and sex-matched controls (from: Harvard Brain Bank; Boston - USA), we investigated, the epigenetic profile. Both immunofluorescence and reduced representation bisulfite sequencing (RRBS) analyses showed that in CDD brains there are profound abnormalities of the epigenetic landscape that impact both histones' modifications and DNA methylation pattern. Moreover, we assessed whether such epigenetic alterations were associated with changes in gene expression. RNA-seq followed by the application of bioinformatics tools for gene expression analysis

revealed robust changes in transcript levels, and myelination-related genes were identified, by gene ontology, as the mostly affected in CDD. Finally, we found that the myeloarchitecture is affected both in the BA17 cortex of CDD individuals and in the V1 cortex of CDKL5-KO mice. Our data, collected on a limited dataset, suggest that CDKL5 is an important player in the regulation of both epigenetic and gene transcription and point to myelination as a novel sign underlying CDD pathogenesis.

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Poster

P063. Developmental Disorders: Molecular and Cellular Mechanisms

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P063.08

Topic: A.07. Developmental Disorders

Support: NIH Grant R01-NS080390
NIH Grant R01-NS095733

Title: Evidence of aberrant rhombic lip development in the pathogenesis of Dandy-Walker malformation

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Abstract: The rhombic lip is a dorsally located germinal zone in the hindbrain that produces ~80% of all neurons in the human brain and assists in the production of all glutamatergic neurons in the cerebellum. While the mouse rhombic lip is a transient structure present between embryonic days 12.5 and 17.5 lacking apparent structural compartmentalization, by contrast, in humans, the rhombic lip is temporally expanded, long-lived and seen throughout gestation. It is also uniquely compartmentalized into a ventricular (RL^{VZ}) and subventricular zone (RL^{SVZ}) separated by a distinct vascular bed. The RL^{VZ} is composed of proliferative self-renewing

SOX2+ cells, while the RL^{SVZ} is made up of progenitors of two main RL derivatives, Granule Cell Precursors (GCPs) and Unipolar Brush Cells (UBCs). The levels of GCPs vs UBC-progenitors changes over time with GCPs produced in higher numbers initially followed by UBCs. The human RL also exhibits human-specific expansion of bi-potential UBC-progenitors. Histopathological and immunohistochemical analyses of developing 31 Dandy Walker Malformation (DWM) and Cerebellar Vermis Hypoplasia (CVH) samples suggest that rhombic lip disruption is associated with cerebellar hypoplasia which disproportionately affects the posterior vermis and is correlated with reduced proliferation and self-renewal of the rhombic lip progenitor pool. Our findings are significant and add to the understanding of the human cerebellar development as well as the developmental pathogenesis of cerebellar birth defects like DWM and CVH.

Disclosures: A.H. Sjoboen: None. **P. Haldipur:** None. **S. Bernardo:** None. **T. Sivakumar:** None. **K.A. Aldinger:** None. **M. Deng:** None. **F. Guimiot:** None. **H. Adle-Biassette:** None. **R. Russo:** None. **J.R. Siebert:** None. **D. Kidron:** None. **F. Razavi:** None. **I.A. Glass:** None. **C. Di Gioia:** None. **E. Silvestri:** None. **K.J. Millen:** None.

Poster

P063. Developmental Disorders: Molecular and Cellular Mechanisms

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Program #/Poster #: P063.09

Topic: A.07. Developmental Disorders

Support: R21 NS104560 (JS)
P50HD103538(AF)
Run for ALD and Brian's Hope Foundation (AF, AM, BT)

Title: Micro-rna and metabolomics signatures for adrenomyeloneuropathy disease severity

Authors: B. TURK[1], L. M. POISSON[2], C. L. NEMETH[1], J. GOODMAN[1], A. MOSER[1], R. JONES[1], A. S. FATEMI[1], ***J. SINGH**[3];
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Abstract: Micro RNA and Metabolomics Signatures for Adrenomyeloneuropathy Disease Severity Turk BR[1], Poisson LM [2] , Nemeth CL[1], Goodman J[1], Moser A[1], Jones RO[1], Fatemi A[1], Singh J[3]
[1] Moser Center for Leukodystrophies, Kennedy Krieger Institute, Johns Hopkins Medical Institutions[2] Department of Public Health Sciences, Henry Ford Hospital[3] Department of Neurology, Henry Ford Hospital
ABSTRACTObjective: Adrenomyeloneuropathy (AMN), the slow progressive phenotype of

adrenoleukodystrophy (ALD) has no clinical biomarker for disease progression. This study aimed to identify plasma biomarkers for AMN disease severity in human blood plasma. Biomarker discovery for AMN severity would facilitate clinical trial placement and fulfill an unmet need in determining disease severity in clinical practice.**Methods:** A cross-sectional discovery study using GC/MS-based metabolomics and RNA-seq based miRNA was performed in human blood plasma. Integrated pathway-, clustering- and regression analyses were performed.**Results:**14 AMN and 6 healthy control subjects were assayed. Categoric comparison betweencontrol, mild, and moderate/severe AMN shows strong metabolic and miRNA clustering signatures. Individual clinical biomarker performance was assessed via regression against patient's clinical Expanded Disability Status Score (EDSS). Strong clinical correlations were shown for: 7-alpha-hydroxy-3-oxo-4-cholestenoate (7-HOCA) ($r2=0.83$, $p<0.00001$), dehydroepiandrosteronesulfate (DHEA-S) ($r2=0.82$, $p<0.00001$), hypoxanthine (0.q82, $p<0.00001$), as well as miRNA-451a ($r2=0.68$, $p<0.00001$) Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis identified downstream systems and pathways as a function of disease progression, by comparing mild vs severe disease: GAREM, IGF-1, CALCRL, SMAD2&3, Glutathione peroxidase, LDH and NOS.**Interpretation:** This is the first report of unique miRNA and metabolic signatures for AMN disease severity. We identify several candidate markers with strong correlations with clinical EDSS, providing potential biomarkers for clinical use and clinical trial. Network analysesidentify and implicate vascular, inflammatory, and oxidative stress pathways altered as a function of disease progression.

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Poster

P063. Developmental Disorders: Molecular and Cellular Mechanisms

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Program #/Poster #: P063.10

Topic: A.07. Developmental Disorders

Support: The Lou Lou Foundation

Title: Dysregulation of central and peripheral microtubule proteins in CDKL5 deficiency disorder (CDD)

Authors: A. THORNTON[1], C. DE PASQUALE[1], C. K. CALLAGHAN[2], ***A. FREEBURN**[2], I. BARBIERO[3], C. KILSTRUP-NIELSEN[3], M. BIANCHI[2];
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Abstract: Background: CDKL5 Deficiency Disorder (CDD) is a rare X-linked brain disorder characterized by severe early-onset seizures, intellectual disability, motor and social impairment. CDD is the result of a heterogenous mutation on the *CDKL5* gene. Microtubules (MT) are cytoskeletal elements formed by the polymerization of α/β-tubulin heterodimers and MT dynamics are fundamental for brain development and are involved in the pathogenesis of CDD. Here, we examined brain and plasma α-tubulin post translational modifications (PTMs) associated with MT dynamics in *Cdkl5*-KO mice and human CDD samples. **Method:** α-tubulin PTMs were measured using Infrared western blot in the hippocampus and plasma of *Cdkl5*-KO (exon 4) male mice compared to age-matched wild-type mice at postnatal day (P) 20, 60 and 180 and in plasma samples of CDD patients (n=11, female; mean age: 11, age range: 2-27) and healthy controls (female n=6 and male n=3; mean age: 39, age range: 27-49). Acetylated-Tubulin (Acet-Tub, less dynamic MT) was normalised to Total-Tubulin (Tot-Tub) and Tyrosinated-Tubulin (Tyr-Tub; dynamic MTs) and Detyrosinated-Tubulin (Glu-Tub; less dynamic MTs) were analysed as a ratio. Data were analysed by two-way ANOVA followed by Fisher's LSD *post hoc* or student's t-test. $P<0.05$ was deemed significant.**Results:** At P180, Acet/Tot-Tub is significantly overexpressed, and Tyr/Glu-Tub is significantly downregulated in the hippocampus of *Cdkl5*-KO mice compared to WT. While not significant, similar trends are evident at P20 and P60. Acet/Tot-Tub tends to be overexpressed in the plasma of *Cdkl5*-KO mice. In line with this latter data, our human results showed a significant plasma Acet/Tot-Tub overexpression in CDD patients compared to controls. This increase suggests similar MT dynamics dysfunction in *Cdkl5*-KO mice and CDD patients. Tyr/Glu-Tub is unaltered in the plasma of CDD patients. **Conclusion:** *Cdkl5*-KO mice and CDD patients exhibit alterations in α-tubulin PTMs consistent with dysregulated MT dynamics during neurodevelopment, indicating that MTs may serve as a novel therapeutic target for CDD. Moreover, plasma Acet-Tub may represent a potential biomarker of disease progression in CDD.**Acknowledgements:** This work is funded by the Lou Lou Foundation.

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Poster

P081. Dopamine Release and Monoamine Transport

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P081.01

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: NSERC RGPIN-2020-05279
Parkinson Canada

Title: The calcium sensor Synaptotagmin-1 is critical for phasic dopamine release, but is dispensable for motor behaviors in mice

Authors: *B. DELIGNAT-LAVAUD[1], J. KANO[1], C. DUCROT[1,2], I. MASSÉ[2], S. MUKHERJEE[1], N. GIGUÈRE[1,3], L. MOQUIN[3,4], C. LÉVESQUE[4], S. BURKE NANNI[1], M.-J. BOURQUE[1], P. ROSA-NETO[3], D. LÉVESQUE[4], L. DE BEAUMONT[2], L.-É. TRUDEAU[1]; [1]Univ. De Montreal, Montréal, QC, Canada; [2]Hôpital du Sacré-Coeur-de-Montréal, Montréal, QC, Canada; [3]The Douglas Res. Centre, McGill Univ., Montréal, QC, Canada; [4]Fac. of Pharmacy, Univ. de Montréal, Montréal, QC, Canada

Abstract: Midbrain dopamine (DA) neurons, a population of cells that are critical for motor control, motivated behaviors and cognition, release DA via an exocytotic mechanism from both their axonal terminals and their somatodendritic (STD) compartment. Some of this release depends on axon potential firing, calcium influx and activation of exocytosis calcium sensors, while other forms can be triggered locally by afferent inputs or occur spontaneously. The mechanisms and physiological roles of these different forms of DA release are unclear. Here we conditionally deleted the synaptotagmin 1 (Syt1) gene in DA neurons (cKO^{DA} mice) to determine the roles of activity-dependent DA release. We found that in these mice, while, evoked axonal DA release is largely abrogated in the striatum and strongly reduced in the ventral mesencephalon, Syt1 cKO^{DA} mice show intact performance in multiple DA-dependent motor tasks, suggesting that activity-dependent DA release is dispensable for basic motor functions. Strikingly, basal extracellular levels of DA in the striatum, measured by microdialysis/HPLC, were unchanged, suggesting that a basal tone of extracellular DA is sufficient to sustain basic movements. We also found multiple adaptations in the DA system in cKO^{DA} mice, similar to those happening at early stages of Parkinson's disease. Overall, while validating the involvement of Syt1 as the critical calcium sensor controlling evoked-DA release, our study reveals the striking resilience of DA-dependent motor functions in the context of a near-abolition of phasic DA release, shedding new light on why extensive loss of DA innervation is required to reveal motor dysfunctions in Parkinson's disease.

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Poster

P081. Dopamine Release and Monoamine Transport

Location: Virtual Only

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Program #/Poster #: P081.02

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: Lundbeck Foundation Grant R266-2017-4331 (UG)
Lundbeck Foundation Grant R276-2018-792 (UG)
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Independent Research Fund Denmark – Medical Sciences Grant 7016-00325B (UG)

Title: Dissecting differential dopamine dynamics in the dorsal and ventral striatum using the genetically encoded sensor dLight 1.3b

Authors: A. L. EJDRUP[1], S. H. JØRGENSEN[1], M. D. LYCAS[1], L. P. POSSELT[1], L. TIAN[2], J. K. DREYER[3], F. HERBORG[1], A. T. SØRENSEN[1], *U. GETHER[1];
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Abstract: Dopamine neurotransmission in the striatal forebrain modulates movement, learning and reward. The transmission dynamics differ from classic, fast neurotransmission as dopamine is thought to mainly exerts its action through extrasynaptic volume transmission, acting exclusively on metabotropic receptors. Interestingly, there is evidence in the temporal dimension for signalling profiles across timescales, ranging from slow changes in basal levels to release-events with sub-second precision. However, a full understanding of extracellular dopamine dynamics has to a large extent been challenged by the methods available, as e.g., microdialysis suffers from poor temporal resolution and fast-scan cyclic voltammetry measurements are difficult to carry out over extended time periods. Here, we employ fiber photometry together with the genetically encoded dopamine biosensor dLight1.3b to concurrently measure fast dopamine dynamics in the mouse dorsal (DS) and ventral striatum (VS). Our data reveal remarkably different transmission dynamics with a rapidly fluctuating signal in the DS, containing both transients and asynchronous release, and a much more slow-paced signal in the VS, consisting of mostly dopamine transients. The release pattern in the two regions correlated, as dorsal dopamine release predicted ventral release at both a sub-second timescale and across tens of seconds. By recording in the two regions during both a novel environment and a reward seeking behavioural paradigm, we observed widely different responses of the regions with the VS signal exhibiting ramp-like release features. VS dopamine was also linked to locomotion, as ultra-slow fluctuations in ventral dopamine levels correlated with likelihood of movement initiation. Finally, we found that blocking the dopamine transporter with cocaine changed the dynamics of both regions and shifted the predictive power of movement to the dorsal region. This highlights the dopamine transporter as a key regulator of dopamine dynamics and match up well with findings of regional differences in expression levels of the protein.

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Poster

P081. Dopamine Release and Monoamine Transport

Location: Virtual Only

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Program #/Poster #: P081.03

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: Lundbeck Foundation R303-2018-3540

Title: Multimodal Detection of Dopamine by Sniffer Cells Expressing Genetically Encoded Fluorescence Sensors

Authors: C. K. HERENBRINK[1], J. F. STØIER[1], W. D. REITH[1], A. DAGRA[2], M. A. C. GREGOREK[1], Y. LI[3], L. TIAN[4], U. GETHER[1], ***F. HERBORG**[1];
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Abstract: Dopamine serves an important role by supporting both locomotor control and higher brain functions such as motivation and learning. Dopaminergic dysfunction is implicated in an equally multidimensional spectrum of neurological and neuropsychiatric diseases. Extracellular dopamine levels are known to be tightly controlled by presynaptic dopamine transporters (DAT), which is also a main target of psychostimulants. Still, detailed data on dopamine dynamics in space and time is needed to fully understand how dopamine signals are encoded and translated into cellular and behavioral responses, and to uncover the pathological effects of disease on dopamine neurotransmission to potentially discover new treatment options. The recently developed genetically encoded fluorescent dopamine sensors enable unprecedented monitoring of dopamine dynamics and have changed the field of *in vivo* dopamine recording. However, the potential of these sensors to be used for *in vitro* and *ex vivo* assays remains unexplored. Here, we demonstrate a generalizable blueprint for making “sniffer” dopamine cells for multimodal detection of dopamine *in vitro* and *ex vivo*. We generated six different sniffer cell lines with inducible expression of dopamine sensors and performed a head-to-head comparison of sensor properties to guide users in sensor selection. In proof-of-principle experiments, we show how the sniffer cells can be applied to measure release of endogenous DA from cultured neurons and striatal slices, and for determining total DA content in striatal tissue. Furthermore, we use the sniffer cells to quantify DAT-mediated dopamine uptake, and AMPH-induced and constitutive DA efflux as a radiotracer free, high-throughput alternative to electrochemical- and radiotracer-based assays. Importantly, the sniffer cells framework can readily be applied to other transmitter systems for which the list of genetically encoded fluorescent sensors is rapidly growing.

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Poster

P081. Dopamine Release and Monoamine Transport

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Program #/Poster #: P081.04

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: NIH Grant DA045284

Title: Decoding the functional role of ventral tegmental area-olfactory tubercle dopamine transmission in methamphetamine-induced behaviors

Authors: *R. BHIMANI[1], C. E. BASS[2], J. PARK[1];
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Abstract: The olfactory tubercle (OT) is a limbic structure located at the ventral most portion of the ventral striatum and receives some of the densest dopamine (DA) innervation in the brain from the ventral tegmental area (VTA). In addition, the OT is only a few hundred micrometers thick in rodents and is positioned within close proximity to many other DA-rich brain areas (e.g. nucleus accumbens, caudate putamen). Increasing evidence has highlighted that DA transmission in the OT may play a critical role in the reinforcing effects of many drugs of abuse. However, due to the anatomical and neurochemical limitations of traditional techniques to selectively modulate and determine VTA-DA signaling in the relatively small OT, very little is known about the functional link between VTA-DA in the OT and its role in mediating aversion, reward and addiction processes. In this study, we integrated immunohistochemical, neurochemical, and pharmacological approaches coupled with chemogenetics to selectively determine and modulate VTA-DA signaling in the OT. Through use of a combinatorial viral targeting system and *in vivo* fast-scan cyclic voltammetry, we restricted designer receptors exclusively activated by designer drugs (DREADDs) to VTA-DA neurons in order to inhibit neuronal activity and monitor real-time DA transmission in awake-behaving rats. Our findings demonstrate selective transfection of DREADDs to VTA-DA neurons in wild-type rats and highlight that chemogenetic inhibition of VTA-DA transmission in the OT inhibits the behavioral and reinforcing effects of methamphetamine (METH) as well as attenuates METH-induced VTA-DA transmission in the OT. These findings will provide a novel understanding of the VTA-DA-OT circuit and how it is involved in processing psychostimulant-induced reward and drug addiction.

Disclosures: R. Bhimani: None. **J. Park:** None.

Poster

P081. Dopamine Release and Monoamine Transport

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Program #/Poster #: P081.05

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: NIH Grant DA035714
NIH Grant DA041932
UofSC SPARC Grant

Title: A Novel Allosteric Dopamine Transporter Modulator, SRI-32743, Reverses HIV-1 Tat Protein-Induced Changes in Extracellular Dopamine Levels in the Caudate Putamen of Inducible Tat Transgenic Mice

Authors: *S. DAVIS[1], M. FERRIS[2], S. ANANTHAN[3], O. MOUKHA-CHAFIQ[3], S. K. SAIN[3], C. E. AUGELLI-SZAFRAN[3], J. ZHU[1];
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Abstract: Inhibition of the dopamine (DA) transporter (DAT) by the HIV-1 protein transactivator of transcription (Tat) has been implicated as a mediating factor of HIV-1 associated neurocognitive disorders (HAND). Our published work has demonstrated that DA uptake through DAT is decreased in the prefrontal cortex of the inducible Tat transgenic (iTat-tg) mouse model. This model recapitulates many neurocognitive impairments observed in HIV infected individuals. Further, our preliminary studies show that the Tat-induced inhibition of DA transport can be attenuated by SRI-32743, a novel DAT allosteric modulator. This study utilized Fast Scan Cyclic Voltammetry (FSCV) to determine the effects of *in vivo* Tat expression on extracellular DA levels and the impact of SRI-32743 on the Tat effect in iTat-tg mice. Following a 7-day administration of doxycycline (Dox, induces Tat expression) or saline, the baseline of tonic (1 Hz) or phasic (20 Hz, 5 pulses) DA release in the caudate putamen was measured. We found a significant increase (~50%) in phasic but not tonic DA release in Dox-treated iTat-tg mice compared to saline control mice. To distinguish the distinct Tat inhibitory effects on DAT and vesicular monoamine transport-2, we investigated whether iTat-tg mice treated with Dox or saline display differential changes in phasic DA levels in response to nomifensine, a selective inhibitor for DAT. Both saline- and Dox-treated iTat-tg mice displayed enhanced phasic DA release in response to nomifensine in a concentration-dependent manner (1 nM-10 μM), in which the nomifensine-induced increase in DA level in Dox-treated iTat-tg mice was about 30-50% greater than that observed in saline control mice. This suggests that Tat expression impairs DAT function, leading to the increased extracellular DA release. Finally, we determined whether systemic administration of SRI-32743 can reverse the Tat-increased phasic DA release. A single

injection of SRI-32743 (10 mg/kg, i.p.) was given to these mice 30 min prior to the mice being sacrificed. Results show that SRI-32743 reversed the increase in baseline of phasic DA release and nomifensine-augmented DA levels in Dox-treated iTat-tg mice, while SRI-32743 alone did not alter baseline of DA release. These findings suggest that *in vivo* exposure of mouse brain to HIV-1 Tat protein increases the extracellular DA levels, in which SRI-32743, through a positive allosteric modulation of DAT, reverses the Tat-mediated changes in the DA levels. Thus, this study demonstrates that developing allosteric modulatory ligands will aid in the drug discovery efforts for therapeutic interventions for HIV-infected patients.

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Poster

P081. Dopamine Release and Monoamine Transport

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Program #/Poster #: P081.06

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: NIH Grant DA035714
NIH Grant DA041932
SC INBRE SIRP Grant
UofSC Honors College SURF Grant
UofSC Magellan Grant

Title: Mutational effects of the human norepinephrine transporter on basal and Tat-induced inhibition of dopamine transport

Authors: *K. D. PORTER[1,3], S. E. DAVIS[1], M. J. STRAUSS[1], Y. YUAN[3], S. LIN[1], P. MCQUILLEN[1], K. H. DAVENPORT[1], C.-G. ZHAN[3], J. ZHU[2];
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Abstract: The HIV regulatory protein, transactivator of transcription (Tat)-induced dysregulation of dopaminergic transmission has been implicated as a central factor in the development of HIV-1 associated neurocognitive disorders (HAND). Our recent report revealed that dopamine (DA) transport through the norepinephrine (NE) transporter (NET) is reduced in the prefrontal cortex of inducible Tat transgenic mice. The prefrontal cortex is an important brain region for higher cognitive function, where the NET plays a critical role in the reuptake of DA and NE. Our recently developed three-dimensional computational model of Tat-human NET (hNET) showed that Tat-hNET binding is highly dynamic and that Tat preferentially binds to

hNET in its outward-open state. HIV-1 Tat forms hydrogen bond interactions with side chain hNET residue threonine544. Our previous study demonstrated that mutation of histidine547 (H547A) on the human DA transporter (hDAT) enhances DA uptake and attenuates Tat-inhibited DA transport. Based on these findings, this study examined the mutational effects of threonine544 on human NET (hNET), the corresponding residue of hDAT histidine547, on basal and Tat-induced inhibition of DA uptake through hNET. Compared to WT hNET, the kinetic property (V_{max}) of [^{3}H]DA uptake in PC12 cells expressing WT hNET and mutants was retained as normal in mutation of hNET threonine544 to alanine (T544A) but reduced in a different substitution, threonine544 to proline (T544P). No change in K_m was found in both mutants relative to WT hNET. Results from biotinylation assay show that expression of total and surface hNET protein was not altered in T544A but reduced in T544P relative to WT hNET. Additionally, we also examined the effects of T544-associated hNET residues Y545, D546, D547, and Y548 on DA uptake by mutating these residues to Y545H, D546L, D547L and Y548H. Compared to WT hNET, the V_{max} values of [^{3}H]DA uptake was retained as normal in Y545H, decreased in D546L, and increased in D547L and Y548H. The K_m values were not altered in Y545H and D546L but increased in D547L and Y548H, suggesting that the increased DA uptake in D547L and Y548H is accompanied with decreased K_m. Further, no changes in IC_{50} values for DA inhibiting [^{3}H]DA uptake were found among these mutants. Our ongoing effort is investigating the effect of these mutants on Tat-induced inhibition of DA uptake through hNET, which will help to further understand the mechanism of Tat interacting with hNET. These current findings provide a novel insight into the molecular basis for developing selective compounds for Tat-NET interactions in the context of HAND.

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Poster

P081. Dopamine Release and Monoamine Transport

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P081.07

Topic: B.01. Neurotransmitters, Transporters, and Signaling Molecules

Support: SNSF grant No. P2BSP3_191740
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Austrian Science Fund/FWF doc.fund DOC33
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Title: (2-Aminopropyl)benzo[β]thiophenes (APBTs) are novel monoamine transporter ligands that lack stimulant effects but display psychedelic-like activity in mice

Authors: D. RUDIN[1], ***H. SITTE**[1], J. MCCORVY[2], G. C. GLATFELTER[3], D. LUETHI[1], D. SZÖLLÖSI[1], T. LJUBIŠIĆ[1], P. KAVANAGH[4], G. DOWLING[4], M. HOLY[1], K. JAENTSCH[1], D. WALTHER[3], S. D. BRANDT[5], T. STOCKNER[1], M. H. BAUMANN[3], A. L. HALBERSTADT[6], *H. H. SITTE[1];
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Abstract: Derivatives of (2-aminopropyl)indole (API) and (2-aminopropyl)benzofuran (APB) are new psychoactive substances which produce stimulant effects *in vivo*. (2-Aminopropyl)benzo[β]thiophene (APBT) is a novel sulfur-based analog of API and APB that has not been pharmacologically characterized. In the current study, we assessed the pharmacological effects of six APBT positional isomers *in vitro*, and three of these isomers (3-APBT, 5-APBT, and 6-APBT) were subjected to further investigations *in vivo.* Uptake inhibition and efflux assays in human transporter-transfected HEK293 cells and in rat brain synaptosomes revealed that APBTs inhibit monoamine reuptake and induce transporter-mediated substrate release. Despite being non-selective transporter releasers like MDMA, the APBT compounds failed to produce locomotor stimulation in C57BL/6J mice. Interestingly, 3-APBT, 5-APBT, and 6-APBT were full agonists at 5-HT_2 receptor subtypes as determined by calcium mobilization assays and induced the head-twitch response in C57BL/6J mice (a 5-HT_{2A}-mediated behavior), which mirrors the actions of known psychedelic drugs. A comparison of APBT compounds with their APB counterparts demonstrated that replacing the oxygen atom with sulfur results in enhanced releasing potency at the serotonin transporter and more potent and efficacious activity at 5-HT_2 receptors fundamentally changing the *in vitro* and *in vivo* profile. Our data suggest that APBT isomers may exhibit psychedelic or entactogenic effects, with minimal psychomotor stimulation. Whether this pharmacological profile translates into potential therapeutic potential, for instance as candidates for drug-assisted psychotherapy, warrants further investigation.

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Poster

P164. Microglia: Disease Mechanisms III

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P164.01

Topic: B.11. Glial Mechanisms

Support: Gwendolyn Lawliss Trust

Kurt and Ingrid Dahrén Foundation

Title: Inflammasome assembly as a potential mechanism of microglial activation and neuroinflammation in schizophrenia

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Abstract: Schizophrenia (SCZ) is a debilitating psychiatric disorder that afflicts around 1.1% of the world population with symptoms including of disorganized thoughts, hallucinations, and lack of emotions. Although[VRT1] the disorder has been studied for decades, the etiology and biological causes underlying SCZ have been difficult to characterize. Studies have proposed that immune-mediated neuroinflammation may be driving the disease onset and early progression. The inflammatory response may be triggered by over activated microglia, the immune cells of the central nervous system that can create a host of inflammatory mediators. One potential inflammatory pathway is the inflammasome complex which is a multi-protein complex that forms in response to the detection of foreign antigens and results in the production of several pro-inflammatory cytokines. These complexes are of interest in SCZ, because their assembly and the cytokines produced by the formation of inflammasome are thought to occur in microglia. In this study we investigate if the inflammasome complex is seen in microglia in donor brains with SCZ. Additionally, we hypothesis that the morphological changes seen in the microglia from postmortem brain samples of SCZ donors can be correlated to an increase in the inflammasome assembly. To investigate if the inflammasome proteins are increased in SCZ we assembled a cohort of postmortem human brains with SCZ and neurotypical controls (N= 13 SCZ; N= 16 control) and prepared sections from the frontal cortex, superior temporal cortex, and the anterior cingulate cortex. The identification of cells expressing the inflammasome protein, apoptosis-associated speck-like protein containing a CARD (ASC), was achieved using immunohistochemical staining performed on donor brains with SCZ and neurotypical controls. Results show the number of cells expressing ASC protein in cases with SCZ increased by 43% in the frontal cortical gray matter and 51% in the superior temporal cortical gray matter in brains with SCZ compared to controls. The results suggest that inflammasome assembly occurs in cases of SCZ and the incidence of assembly may be region specific. These results suggest that the inflammasome complex may be pathologically important in SCZ and may serve as a future therapeutic target that could lead to the development of novel and much needed treatment strategies.

Disclosures: R. Gober: None. **A. Barreda:** None. **S.P. Garamszegi:** None. **X. Sun:** None. **W. Scott:** None. **R. Vontell:** None. **L. Duque:** None.

Poster

P164. Microglia: Disease Mechanisms III

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P164.02

Topic: B.11. Glial Mechanisms

Support: Faculty start-up funds to LKF

Title: Microglia-specific knockdown of the circadian clock gene Bmal1 reduces anxiety and alters LPS-induced neuroinflammation in a time-of-day and sex-specific manner

Authors: *L. M. INCE, L. K. DAVIS, L. K. FONKEN;
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Abstract: Microglia are key cells regulating neuroimmune function, learning & memory, and behavior. Microglia activity varies throughout the day, driven by the animal's circadian rhythm which is itself entrained by external factors such as light-dark cycles and food availability. This daily cycle is evident in microglia inflammatory responses, with microglia isolated during the rest phase (light/day) producing more pro-inflammatory cytokines after LPS stimulation than microglia isolated during the active phase (dark/night). However, it remains unclear to what extent this is driven by the cell-intrinsic clock within microglia themselves versus cell-extrinsic cues. To analyze the effects of the cell-intrinsic circadian clock on microglia function, we generated mice with microglia-specific knockdown of the core clock gene Bmal1 ($Bmal1^{flox/flox}Tmem119^{CreERT2}$, microglia-$Bmal1^{KD}$) and performed a battery of baseline behavior tests along with *in vivo* and *in vitro* inflammatory challenge. Compared with Bmal1-proficient littermates ($Bmal1^{flox/flox}$), male microglia-$Bmal1^{KD}$ mice showed increased activity in the open field test, with greater time spent moving and increased distance travelled. Females were more active than males but did not show a genotype effect. This pattern of hyperactivity was also observed in the elevated plus maze, with male microglia-$Bmal1^{KD}$ mice moving significantly further than wild type littermates, and showing a trend for increased time spent in open arms. This may indicate that Bmal1 in microglia plays a role in anxiety-like behavior in male mice, but not in females. In contrast, no genotype effects were observed in novel object recognition or in sucrose anhedonia. We next tested the impact of microglia-$Bmal1^{KD}$ after peripheral inflammatory challenge by injecting mice with LPS (0.5mg/kg i.p.) during the middle of their rest phase (mid-light) or the middle of their active phase (mid-dark) and collected tissue samples 3 hours later. Whilst wild type females showed time-of-day variation in both *Il6* and *Il1b* mRNA expression in the hippocampus, with higher expression during the rest phase, microglia-$Bmal1^{KD}$ females showed a rhythm in *Il6* but not in *Il1b*. These results implicate microglial Bmal1 in regulation of behavior and neuroinflammation in a time-of-day and sex-specific manner. Future work will determine: 1) how the sex-specific effects of microglia-$Bmal1^{KD}$ in anxiety-like behavior are generated; 2) how sickness behavior is modulated by microglia-$Bmal1^{KD}$; and 3) how Bmal1 modulates additional microglial functions such as phagocytosis, autophagy, synaptic pruning, and vesicular exocytosis.

Disclosures: L.M. Ince: None. **L.K. Davis:** None. **L.K. Fonken:** None.

Poster

P164. Microglia: Disease Mechanisms III

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Program #/Poster #: P164.03

Topic: B.11. Glial Mechanisms

Support: NIDA 5T32DA007278
NIDA 5R01DA041356

Title: Microglia respond to extracellular ATP release during opioid withdrawal by increasing intracellular calcium via P2X4 and P2X7 purinergic receptors

Authors: *D. BERGKAMP[1], J. EL KHOURY[3], G. D. STUBER[2], J. F. NEUMAIER[4];
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Abstract: Neuroimmune activation in the central nervous system during opioid withdrawal occurs in conjunction with major changes in activity of neural circuits. Microglia respond to changes in neuronal firing rates by releasing inflammatory factors such as interleukin-6 and TNF-alpha. These signaling molecules contribute to dysphoria and the unpleasant experiences of withdrawal, especially in structures such as the striatum, which are essential to animal motivation and habitual behaviors. We hypothesize that extracellular ATP released during opioid withdrawal increases intracellular microglial calcium and initiates neuroimmune signaling to address abnormal synaptic plasticity associated with addiction.
We prepared coronal brain slices including striatum from male and female mice following five days of fentanyl administration and naloxone precipitated-withdrawal, and used two photon imaging to visualize changes in fluorescence of two biosensors: virally expressed iATPSnFR1 to measure extracellular ATP or GCaMP6f expressed selectively in microglia, using a transgenic strategy, to observe changes in calcium dynamics. We are currently testing whether precipitated opioid withdrawal rapidly increases extracellular ATP in striatum using iATPSnFR1. Additionally, we predict that either bath-applied ATP or naloxone-precipitated withdrawal increases the rate and magnitude of calcium influx events in microglia in the striatum. All statistical analyses are performed with custom written scripts to run ANOVA and tests for adjusted p-values for multiple comparisons where appropriate. In future experiments, we will investigate whether incubation of slices with P2X7 or P2X4 purinergic receptor antagonists is sufficient to block changes in intracellular microglial calcium occurring during opioid withdrawal. These complex neuroimmune signaling events may indicate that microglia are a promising therapeutic target for mitigating withdrawal and reducing the propensity to relapse to opioid seeking.

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Poster

P164. Microglia: Disease Mechanisms III

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Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P164.04

Topic: B.11. Glial Mechanisms

Support: FRM Grant FDT202106012955

Title: Protective role of Microglia and T cells in a mouse model of temporal lobe epilepsy

Authors: *M. MORENO-MONTANO[1], P. NOBILI[1], L. MARTINS[1], J. MATEOS-LANGERAK[2], A. VIRENQUE[1], V. DARDALHON[3], H. HIRBEC[1], V. GARCIA[1], N. MARCHI[1], E. AUDINAT[1];
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Abstract: Protective role of microglia and T lymphocytes in a mouse model of temporal lobe epilepsy. Temporal lobe epilepsy (TLE) is a chronic neurological disease affecting brain structures implicated in cognitive functions. The disease is manifested by the appearance of disabling, spontaneous, recurrent seizures caused by the hyper-excitability and hyper-synchronization of a more or less extensive group of neurons in the temporal lobe structures. Seizures are accompanied by neuronal death, gliosis and structural and synaptic reorganization. Sixty percent of patients do not respond to anti-epileptic and the only curative intervention is the surgical resection of the epileptic focus. It is therefore urgent to better understand the mechanisms involved in pathogenesis in order to identify new therapeutic targets.It is now well documented that neuroinflammation is critically involved in this disease. Accumulated data suggest that microglia develop a dual profile, pro and anti-inflammatory, and that the balance between these two states determines the evolution of the disease. Using a TLE mouse model lacking T cells (CD3 -/- mouse), we observed a loss of the microglial anti-inflammatory response and an increase of neuronal death in the hippocampus together with an increase of spontaneous seizure frequency. Regulatory T cells (Treg) have been shown to promote anti-inflammatory reactions in different models of CNS disorders. In preliminary experiments we therefore depleted Treg by administrating anti-CD25 antibodies during early epileptogenesis and we observed a loss of the microglial anti-inflammatory response and an increased neuronal death. We will confirm these results and test whether this treatment alters the development of seizure events. Our working hypothesis is that, interactions between microglia and T cells drive a protective anti-inflammatory effect in the epileptic hippocampus. We will therefore assess whether microglial

depletion phenocopies Treg depletion. These findings may allow the identification of new therapeutic targets to enhance the endogenous anti-inflammatory response and ultimately improve the treatment of TLE.

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Poster

P164. Microglia: Disease Mechanisms III

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P164.05

Topic: B.11. Glial Mechanisms

Title: Inhibiting CSF1 receptor signaling dampens microglial proliferation and reduces Tau deposition in mice

Authors: C. BRIFAULT, L. FOURGEAUD, A. BOTTELBERGS, S. ALLHANDS, H. KIM, L. ZHOU, P. TE RIELE, R. GUPTA, *A. BHATTACHARYA;
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Abstract: Microglia are the resident immune cells of the central nervous system. They maintain brain homeostasis by eliminating pathogens, dead cells, toxins and remodeling synapses. In part, they rely on colony-stimulating factor-1 receptor (CSF1R) signaling to proliferate, activate and self-renew, in both health and disease. Proliferation and activation of microglia around amyloid plaques is an eminent feature of Alzheimer's disease (AD). CSF1R is a tyrosine kinase activated by CSF1 and IL-34. CSF1 is produced by astrocytes, oligodendrocytes and microglia while neurons express IL-34. Reduced plasma levels of CSF1 predicts rapid progression of mild cognitive impairment to dementia. Amyloid activated microglia proliferate producing cytokines like TNFα that worsens neuroinflammation. In preclinical models of AD, microglia depletion has been associated with reduced inflammation, decreased amyloid and Tau pathology and reduced cognitive dysfunction. Mice with conditional microglial CSF1R KO showed elevated expression of IL-34, TREM2, and β-Catenin genes associated with improved cognition and reduced Aβ burden. Here we tested selective brain penetrant small molecule CSF1R antagonist: JNJ-40346527 (JNJ527) to inhibit microglia proliferation. In human iPSC-derived microglia JNJ527 dose-dependently inhibits CSF1/IL34 induced proliferation. *In vivo*, CSF1R inhibition reduced microglia proliferation following LPS challenge. We also tested JNJ-527 in preclinical models of Alzheimer's disease including the APP/PS1 (amyloid) and the human Tau P301S model (Goedert) that recapitulate several molecular and cellular features of human Tauopathies. Animals that received JNJ527 show reduced microglia proliferation (cell number, Ki67 labeling, scRNAseq) compared to vehicle. Inhibition of microglia proliferation correlated with reduction

of pathology including microglia activation (morphology), inflammation (cytokines) and deposition of hyperphosphorylated Tau. CSF1R inhibition with JNJ-527 also resulted in elevation of CSF1 and IL34 in CSF of mice. These ligands can potentially serve as target engagement biomarkers for clinical investigation of CSF1R inhibitors. Overall, these findings strongly support the inhibition of CSF1R to reduce microglial proliferation as a promising immunomodulatory strategy for the treatment of AD.

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Poster

P164. Microglia: Disease Mechanisms III

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Program #/Poster #: P164.06

Topic: B.11. Glial Mechanisms

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Title: Microglia mediate early corticostriatal synapse loss in Huntington's Disease through complement-dependent mechanisms

Authors: *D. K. WILTON[1], K. J. MASTRO[1], M. D. HELLER[1], F. W. GERGITS[1], C. WILLING[1], A. FROUIN[1], A. DAGGETT[3], X. GU[4], A. Y. KIM[1], R. L. FAULL[5], S. JAYADEV[6], X. W. YANG[7], B. A. STEVENS[2];
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Abstract: Synaptic dysfunction is becoming increasingly recognized as a common pathological event in many neurodegenerative diseases, including Huntington’s disease (HD). Despite HD being a monogenetic disease with a well characterized pathology, the molecular mechanisms underlying synaptic dysfunction remain poorly understood. In this study, we show that loss of specific synaptic connections can be observed prior to the onset of motor or cognitive

impairment in multiple slow progressive Huntington's disease mouse models and that microglia contribute to this via a complement-dependent mechanism.
During development, synapse loss as a result of pruning is a normal and highly regulated process required for the correct wiring of the brain. Microglia together with complement proteins, a key component of the innate immune system, play a key role in regulating this process (Stevens et al, 2007; Schafer et al., 2012; Paolicelli et al., 2011). Here we provide evidence that this developmental mechanism is aberrantly reactivated in Huntington's disease mouse models leading to loss of specific synaptic inputs. Using super resolution imaging, genetic ablation, viral-mediated labeling and slice electrophysiology, we demonstrate that complement proteins selectively target corticostriatal connections for engulfment by microglia and show that this process requires mutant huntingtin expression in both striatal and cortical neurons. Moreover, strategies to block this mechanism using genetic or antibody mediated approaches are able to not only prevent loss of synaptic structures and restore excitatory input to the striatum but also rescue early deficits in visual discrimination learning and reversal touchscreen tasks. Together these results demonstrate that elements of the innate immune system, previously only thought to be involved in late stage inflammation, mediate the loss of specific synaptic connections early in Huntington's disease pathology.
Notably, we find that aspects of this synaptic elimination mechanism can also be observed in human disease, as post-mortem tissue from Huntington's disease patients reveals evidence of synapse loss, complement deposition at synapses and phagocytic microglia. Moreover, complement protein levels in the CSF of premanifest HD patients correlated with measures of disease burden. We're currently building on these findings to develop additional fluid and imaging biomarkers that can hopefully be used to predict symptom onset as well as other aspects of clinical progression.

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Poster

P164. Microglia: Disease Mechanisms III

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Program #/Poster #: P164.07

Topic: B.11. Glial Mechanisms

Support: Ruby and Perry Stevens Parkinson's Disease Center of Excellence
NIA K01 AG066747
NARSAD Young Investigator Award
UTHSCSA Center for Biomedical Neuroscience

Title: Loss of the L-type calcium channel Cav1.2 in microglia is protective in the MPTP model of Parkinson's disease

Authors: S. SMITH, J. WICKLINE, A. M. WELLS, ***S. C. HOPP**;
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Abstract: Microglia activation is a noted feature of Parkinson's disease (PD) pathology. Microglia phenotype changes occur early during PD and may be an initiating factor in disease pathology. One driver of microglia phenotype is intracellular calcium. Cohort studies suggest that L-type calcium channel (LTCC) antagonists reduce the odds of developing PD. Studies in PD mouse models suggest that LTCC antagonists protect dopaminergic neurons of the substantia nigra (SN) by reducing age-associated neuronal intracellular calcium oscillations and subsequent mitochondrial stress. We previously reported that the LTCC antagonist nimodipine did not reduce loss of tyrosine hydroxylase (TH) in the SN but did improve motor deficits and reduce microglia activation in the SN in a lipopolysaccharide (LPS)-induced rat model of chronic neuroinflammation (Hopp et al., 2014, J Neuroimmune Pharmacol). Thus, LTCC antagonists may protect neurons from PD pathophysiology by directly modulating microglia intracellular calcium and microglia phenotype. To test this, we first confirmed that the BV2 mouse microglia cell line expresses LTCCs by measuring Cav1.2 and Cav1.3 gene expression. We then tested whether LTCC antagonists directly modulate microglia phenotype by treating BV2 microglia with LPS and increasing doses of the LTCC antagonists isradipine, nimodipine, verapamil, and diltiazem. We found that LTCC antagonists dose-dependently reduced BV2 microglia activation as measured by reduced gene expression of the pro-inflammatory markers IL1β, TNFα, and iNOS. Interestingly, we found that BV2 microglia increased Cav1.2 gene expression after LPS treatment, suggesting that microglia activation induces Cav1.2 expression. To test whether the LTCC Cav1.2 contributes to microglia activation and neurotoxicity, we generated CX3CR1CreERT2$^{+/-}$Cav1.2$^{fl/fl}$ mice with inducible conditional knockout (iCKO) of Cav1.2 on microglia following tamoxifen induction of Cre. Cav1.2$^{fl/fl}$ controls and iCKO experimental mice were induced with tamoxifen and then treated with MPTP to induce neurotoxicity in the SN. We found that Cav1.2 knockout altered microglia and TH immunoreactivity in the SN and modulated motor behavior performance in the open field and rotarod tests. Overall, these data demonstrate that microglia express the LTCCs Cav1.2 and Cav1.3, that LTCC antagonists directly reduce microglia activation *in vitro*, and that Cav1.2 contributes to microglia activation *in vivo*. This suggests that the neuroprotective action of LTCC antagonists may be at least in part via direct modulation of microglia phenotype via action of LTCCs such as Cav1.2 and Cav1.3.

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Poster

P164. Microglia: Disease Mechanisms III

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Program #/Poster #: P164.08

Topic: B.11. Glial Mechanisms

Support: RF1 AG060748
Salk Women and Science award

Title: Microglia TAM receptors are modulators of pathology in Amytrophic Lateral Sclerosis

Authors: *Y. HUANG, G. E. LEMKE;
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Abstract: Amyotrophic Lateral Sclerosis (ALS) is an irreversible and uniformly fatal neurodegenerative disease characterized by selective degeneration of motor neurons (MNs) and progressive muscle dystrophy that is accompanied by chronic neuroinflammation. Reactive microglia are thought to play crucial roles in ALS pathogenesis by mediating the death of MNs and the secretion of pro-inflammatory agents. These cells, among all tissue resident macrophages, express the TAM receptor tyrosine kinases Mer (gene name *Mertk*) and Axl. Our lab and others have previously demonstrated important roles of TAM receptors in microglia and other immune sentinels, including the phagocytic clearance of apoptotic cells and the feedback inhibition of the innate immune response. Recent transcriptomic evidence identifies Axl as part of a small group of 'disease-associated microglia' genes in the $SOD1^{G93A}$ mouse model of ALS. These findings notwithstanding, whether and how microglial TAM receptors participate in ALS disease progression has yet to be explored experimentally. We found a persistent up-regulation of both microglial Mer and Axl, as well as their ligand Gas6, in the $SOD1^{G93A}$ spinal cord beginning at symptomatic onset, suggesting a strong activation of the TAM signaling pathway throughout disease. Interestingly, $SOD1^{G93A}$ mice in the absence of Axl and Mer have significantly advanced symptom onset, but their disease course is almost twice the length of that of TAM-expressing $SOD1^{G93A}$ mice. We showed this delayed arrival of end-stage of disease may be accompanied by dampened microglial activation and reduction in the loss of ALS-vulnerable MNs in the spinal cord, as well as a partial rescue of neuromuscular junction loss and preservation of muscle fiber in the skeletal muscle that may be mediated by TAM deficiency in muscle-resident macrophages. Our work demonstrates a critical and differential involvement of TAM receptors in early and late stages of ALS in both the central and periphery nervous system. Given that TAM inhibitors are already in clinical trials as cancer therapeutics, discoveries from our studies will not only further our understanding of how the innate immune system mediate ALS pathology through TAM signaling, but may potentially be translated into novel therapeutic targets for the treatment for ALS and other neurodegenerative diseases.

Disclosures: Y. Huang: None. **G.E. Lemke:** None.

Poster

P164. Microglia: Disease Mechanisms III

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P164.09

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NINDS R01NS121144
UCSF Department of Anesthesia

Title: Peripheral nerve injury induced microglia proliferation involves two distinct phases

Authors: *P. SU[1], W. TAN[1], J. LEFF[1], Z. GUAN[2];
[2]Pain Mgmt. Ctr., [1]UCSF, San Francisco, CA

Abstract: Introduction: Adult microglia proliferate to self-renew at baseline, and to expand their population upon stimulation. Microglia proliferation is hallmark to a myriad of neurological disorders, including nerve injury; More importantly, inhibition of microglia proliferation attenuates the development of neuropathic pain, indicating the critical contribution of microglia proliferation in the pathogenesis. However, the underlying mechanism is largely unknown.
Methods: All animal research protocols were approved by the institutional animal care and use committee. We crossed *Cx3cr1-yfp-CreER* mice with *Myc*^{fl} or *Tnfaip3*^{fl} mice to genetically delete the respective genes from microglia. Sciatic nerve transection was used as the model for peripheral nerve injury. Single cell RNA sequencing (scRNA-Seq): Single microglia cells from lumbar spinal cord were isolated by fluorescence-activated cell sorting (FACS) and processed for single cell RNA sequencing using 10X Genomics platform. Flow cytometry: Dissociated single cells from spinal cord were stained for cell viability, specific microglia marker (CX3CR1), and Ki67 and analyzed by a flow cytometer. Gene Expression is evaluated by quantitative RT-PCR (qRT-PCR) of RNA isolated from FACS sorted microglia.
Results: Our scRNA-Seq reveals that the time course of microglia proliferation can be divided into early and late phases, corresponding to our qRT-PCR results of early and transient *Myc* upregulation and prolonged *Tnfaip3* downregulation in spinal lumbar cord microglia after nerve injury. Our flow cytometry results demonstrate that deletion of *Myc* specifically abolished the early microglia proliferation 2 days after nerve injury. On the other hand, downregulation of *Tnfaip3* regulates late phase of proliferation day 3 and beyond after nerve injury; and our scRNA-Seq shows that *Tnfaip3* deletion in naïve animals resulted in transcriptomic signature similar to late phase nerve injured animals with increased baseline microglia proliferation. In addition, *Myc* and *Tnfaip3* expression returned to baseline at the termination of microglia proliferation, and over-expression of *Myc* or deletion of *Tnfaip3* prevented the cessation of microglia proliferation.
Conclusions: Microglia proliferation after nerve injury consists of an *Myc* upregulation-mediated early and a *Tnfaip3* downregulation-mediated late phase; and termination of microglia proliferation requires the normalization of *Myc* and *Tnfaip3* expression.

Disclosures: P. Su: None. **W. Tan:** None. **J. Leff:** None. **Z. Guan:** None.

Poster

P164. Microglia: Disease Mechanisms III

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P164.10

Topic: B.11. Glial Mechanisms

Support: NS100294
R01NS114130

Title: Identification of Kv1.3 channel interacting proteins using proximity labeling

Authors: *C. BOWEN[1], T. DOWLING[1], P. BAGCHI[1], H. M. NGUYEN[2], H. WULFF[2], N. T. SEYFRIED[1], S. RANGARAJU[1];
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Abstract: Alzheimer's disease (AD) is a devastating neurodegenerative disease characterized by progressive neuronal loss, accumulation of amyloid-beta(Aβ) plaques, and neuroinflammation. Microglia, the immune cell of the brain, transition to disease associated microglia (DAM) in AD.A subset of DAM, known as proinflammatory DAM, promote neuronal injury, release proinflammatory cytokines, fail to phagocytose Aβ effectively, and adopt a glycolytic metabolic signature. Our lab established that proinflammatory DAM highly express Kv1.3 potassium channels, which regulate potassium efflux, membrane potential, and calcium signaling. Our studies show Kv1.3 blockade reduces immune responses in mouse models of AD. Intracellular domains of Kv1.3 channel subunits contain motifs that could interact with proteins associated with immune signaling pathways, however there is little evidence for this. This study aims to identify unique molecular interactors of Kv1.3 channels in mammalian cells using proximity labeling by the biotin ligase, TurboID, which biotinylates proteins within a 10nm radius. We fused TurboID to N- or C-terminus Kv1.3 using a 15 amino-acid inert linker and transfected the constructs into HEK293 cells. TurboID not fused to Kv1.3 and sham transfection acted as controls. Flow cytometry and patch-clamp electrophysiology confirmed the presence of functional Kv1.3 channels on the cell-surface. Biotinylated proteins were streptavidin bead affinity-enriched from cell lysates and confirmed using western blots. Using label free quantitation mass spectrometry (LFQ-MS), we identified 2,195 biotinylated proteins of which very few were enriched in negative controls. There were 313 proteins as N-term interactors, 385 as C-term interactors, and 226 proteins common to both N and C term fusions, potentially representing proteins indirectly associated with Kv1.3 channels. we identified 14 unique N-term interactors (including metabolic proteins eg. Naca, Txnl1, Stub1, Dnaja1) and 38 unique C-term interactors (including membrane trafficking proteins, eg. Ank3, Plekha7, Erlec1, Tp53bp2). We packaged these plasmids into lentiviruses to transduce into mouse microglial cell lines. Our findings will identify novel molecular mechanisms of immune regulation by Kv1.3 channels in microglia.

Disclosures: C. Bowen: None. **T. Dowling:** None. **P. Bagchi:** None. **H.M. Nguyen:** None. **H. Wulff:** 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.; RO1NS100294. **N.T. Seyfried:** 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.; Grant#s: R01AG070937-01, R01AG071587-01, 3P30AG066511, 5U01AG061356,1P01NS084974, 5R01AG015819 ,1U54AG065187 , R01MH117292 , R01AG061800, 1RF1AG062181, 1U01AG061357. **S. Rangaraju:** 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.; Grant #1-K08 NS099474-01, Grant# R01NS114130, Grant# 1RF1AG071587-01, Grant#1R01NS115994-01A1.

Poster

P164. Microglia: Disease Mechanisms III

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P164.11

Topic: B.11. Glial Mechanisms

Support: NIH R01 NS100294

Title: The voltage-gated K^+channel Kv1.3 as a target for modulating neuroinflammation

Authors: *H. M. NGUYEN, Y.-J. J. CHEN, R. LEE, Y. CUI, R. H. IBRAHIM, H. WULFF; Pharmacol., Univ. of California, Davis, CA

Abstract: Chronic neuroinflammation contributes to the pathogenesis of many neurodegenerative, neuropsychiatric, and neurodevelopmental diseases. However, approaches aiming to relieve neuroinflammation should only target the secondary inflammatory damage without compromising beneficial immune functions. Microglia are the principal macrophages of the brain responsible for the maintenance of homeostasis during normal physiology. Microglia not only can rapidly initiate but can also prolong neuroinflammation in response to pathogenic insults. During microglia activation, many membrane receptors and ion channels undergo significant expression changes. The voltage-gated potassium channel Kv1.3 is an effective regulator of membrane potential and is overexpressed in activated human and rodent microglia associated with numerous inflammatory conditions. We and others have demonstrated that blocking Kv1.3 significantly inhibits microglial activation, proliferation, and production of the inflammatory mediators IL-1β, TNF-α, IL-6, and NO. Blocking microglial Kv1.3 also inhibits components of the inflammatory pathway, including the activation of NFκ-B, p38 MAPK, and the NLRP3 inflammasome. The mechanism underlying this anti-inflammatory action of Kv1.3

inhibition in microglia involves, in parts, the disruption of membrane calcium influx associated with activation of the purinergic P2X4 receptors. In vivo treatment with Kv1.3-selective inhibitors prevent inflammation in multiple preclinical rodent models of neurodegenerative diseases. Here, we show that the genetic deletion or pharmacological inhibition of Kv1.3 with the selective small-molecule PAP-1 improve neurological deficits, reduce infarct size, and brain levels pro-inflammatory cytokines in both mouse and rat ischemic stroke models. We further show that deleting Kv1.3 is also effective in aged mice and mice of both sexes. Importantly, blocking Kv1.3 does not affect phagocytosis of neuronal debris or levels of IL-10 and brain-derived nerve growth factor. Thus, our findings suggest that selective targeting of $Kv1.3^{high}$ microglia is an attractive therapeutic approach that fulfills the criteria required of an immunomodulator to not act as a general immunosuppressant for treating neuroinflammation.

Disclosures: H.M. Nguyen: None. **Y.J. Chen:** None. **R. Lee:** None. **Y. Cui:** None. **R.H. Ibrahim:** None. **H. Wulff:** None.

Poster

P164. Microglia: Disease Mechanisms III

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P164.12

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

Support: NIA R01 AG057525
Rochester Center for Alzheimer's Disease (RCADR)

Title: Neuroinflammatory Mechanisms in a Sterile Acute Lung Injury Model

Authors: *H. LI[1,2], J. DAVID-BERCHOLZ[5], A. I. CACERES[5], N. TERRANDO[5], H. A. GELBARD[1,2,3,4];
[1]Ctr. for Neurotherapeutics Discovery, [2]Dept. of Neurol., [3]Dept. of Neurosci., [4]Dept. of Microbiology and Immunol., Univ. of Rochester Med. Ctr., Rochester, NY; [5]Dept. of Anesthesiol., Duke Univ., Durham, NC

Abstract: With the advent of vaccines for SARS-CoV-2, attention has turned towards “long haulers”, those that have survived the acute infection but are still experiencing a spectrum of symptoms. One of the most significant being the neurological symptoms such as brain fog, delirium, and fatigue. Without a therapeutic intervention, COVID syndrome may soon become a significant burden on the healthcare system. Although the etiology of COVID syndrome is unknown, there has been inconsistent data regarding the possibility of direct neuro-invasion. As such we believe that COVID syndrome may be the result of lung injury and cytokine storm. In line with this theory, we developed a sterile lung injury model utilizing repeated intranasal administrations of lipopolysaccharide (i.n. LPS). We found that repeated i.n. LPS causes a robust

acute lung injury (ALI) particularly an elevation of bronchoalveolar lavage fluid (BALF) protein and BALF leukocytes (neutrophils, lymphocytes, and macrophages) at 24h after the last dose. We then assayed for neuroinflammatory effects at 6h, 24h, and 72h after the last LPS dose. We found that brain endothelial cells are activated acutely, but breakdown of the blood-brain-barrier (BBB) and peripheral leukocyte infiltrates were chronically elevated up to 72h after the last dose. Further, we see a robust elevation of microglial phagocytic and activation markers up to 72h after the last dose of LPS. This in turn causes degeneration of CA1 hippocampal synapto-dendritic architecture. We believe these results provide a framework for a mechanism of neuroinflammation and degeneration as a result of ALI, particularly those living with COVID syndrome. Further, this study opens the potential for therapeutic strategies to alleviate neurological symptoms.

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Poster

P184. Molecular Mechanisms: Brain Aging, Glia and Neuroinflammation

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P184.01

Topic: C.01. Brain Wellness and Aging

Support: FL DOH 8AZ16
U01AG046139

Title: Deletion of the Abi3 locus influences age-progressive amyloid β and tau pathologies in distinctive ways

Authors: K. IBANEZ[1], K. N. MCFARLAND[2], M. ALLEN[3], L. ZOBEL[1], E. GONZALEZ DE LA CRUZ[1], S. SHAH[1], Q. VO[1], C. LESSARD[1], D. RYU[1], C. C. FUNK[4], N. ERTEKIN-TANER[3], T. E. GOLDE[5], *P. CHAKRABARTY[1];
[1]Univ. of Florida, Gainesville, FL; [2]Dept. of Neurol., McKnight Brain Institute, Univ. of Florida, Gainesville, FL; [3]Mayo Clin., Jacksonville, FL; [4]Inst. For Systems Biol., Seattle, WA; [5]Dept. of Neurosci., Col. of Medicine, Univ. of Florida, Gainesville, FL

Abstract: The recently identified S209F variant of the immune gene *ABI3* increases the risk for Alzheimer's disease (AD). How ABI3 is related to AD pathophysiology is unknown. We used a constitutive *Abi3* knock out mice to study the effects of microglial gene deletion on AD-typical Aβ pathology and tau pathology. In this mouse model, velocigene mediated knock-down of the *Abi3* locus resulted in deletion of *Abi3* gene and its overlapping neighboring gene, *Gngt2*. We first confirmed that the sequence alignment of the *Abi3* and Gngt2 genes is conserved among mice and humans. Secondly, using the AMP-AD consensus datasets and 24 independent mouse

datasets, we confirmed that Abi3 and Gngt2 expression changes were concordant. This shows that expression of *Abi3* and *Gngt2* are strongly correlated and thus these genes are co-regulated. We then went on to examine the effects of *Abi3* locus deletion in APP mice. Both partial and complete deletion of *Abi3* locus reduced Aβ levels in young *APP* mice. RNAseq analysis revealed that these mice, in spite of reduced Aβ, displayed gene expression patterns reminiscent of damage associated microglial phenotype. Next, using somatic brain transgenesis technology to overexpress an aggregation prone human tau construct, we found that *Abi3*-/- mice showed exacerbated tau phosphorylation, NFT pathology and astrocytosis. Our data shows that loss of *Abi3/Gngt2* differentially affects gliosis in a gene-dosage and age-dependent manner such that this leads to initial beneficial effect on Aβ pathology but deleterious effect on tau aggregation and Aβ-associated gene signatures. We also found that the recombinant S209F mutant ABI3 protein was phosphorylated to a lower extent compared to wild type ABI3. Further, mutating the Phe at position 209 to an Ala or Asp reverses this deficit in phosphorylation to levels similar to WT protein. Since, phosphorylation of Abi family of proteins is related to their stability and function, this would imply that the Ser to Phe mutation could render the ABI3 protein inactive. Together, this suggests that the AD-associated *ABI3* variant likely represents a loss-of-function mutation associated with dysregulated immunity.

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Poster

P184. Molecular Mechanisms: Brain Aging, Glia and Neuroinflammation

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P184.02

Topic: C.01. Brain Wellness and Aging

Support: T32AG061892
RF1AG057933-01A1
McKnight Brain Institute

Title: Differential effect of tau seeding based on Apolipoprotein E isoform

Authors: *T. M. L. WILLIAMS[1], A. J. RUIZ[1], G. ANTEZANA[1], E. GONZALEZ DE LA CRUZ[1], P. SULLIVAN[2], D. R. BORCHELT[1], P. CHAKRABARTY[1];
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Abstract: Alzheimer's disease (AD), neuropathologically characterized by amyloid β (Aβ) plaques and tau neurofibrillary tangles (NFT), is the most prevalent neurodegenerative disease. The major risk factor for sporadic AD is the presence of the E4 allele of the gene coding for

Apolipolipoprotein E (APOE). The E3 allele in considered the neutral allele whereas the E2 allele is protective for AD. While presence of the E4 allele exacerbates Aβ pathologies clinically and in rodent models, the relative roles of the different APOE alleles in tau aggregation pathways is less clear. In this study, we first examined if APOE affects lifespan in a mouse model of tauopathy. Using the PS19 model of tauopathy that gets paralyzed by 12 months of age, we found that human APOE heterozygous mice had variable median lifespans ranging from 335 days to 372 days (E3<E2<E4) with sex-based differences. PS19 mice homozygous for human APOE alleles showed a trend for early death for APOE4 mice at 317 days (E4<E2<E3). All PS19/APOE mice showed robust tauopathy at end-stage with no clear differences in tau immunoreactivity patterns. We next analyzed whether presence of a specific APOE allele increases the propensity of tau prions to induce pathology at younger ages of these PS19 mice. Using unilateral K18 tau aggregate injections in the hippocampus of 2.5-month-old PS19 mice humanized for APOE, we show that APOE3 mice presented significantly higher burden of tau pathology in the ipsilateral and contralateral hippocampus ($p<0.01$) and cortex ($p<0.05$) (n=6-8 mice). At the age of analysis (7.5 months), the sham-seeded mice had little to no tau pathology. The Iba-1 immunoreactivity followed the trends shown by increased tauopathy in APOE3 mice, while astrocytosis levels remained unchanged among the three APOE mouse lines. Additionally, K18 aggregate injected PS19 mice with endogenous Apoe allele displayed hippocampal tauopathy that was significantly lower than the PS19 mice carrying the human APOE3 allele, suggesting functional differences. Thus, our study shows that human APOE3 increases the propensity of tau seeding, which is concordant with the higher prevalence of primary age-associated tauopathy cohorts carrying E3 allele. The significance of our data, vis-à-vis data from clinical case studies and previously reported rodent models, will be discussed.

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Poster

P184. Molecular Mechanisms: Brain Aging, Glia and Neuroinflammation

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P184.03

Topic: C.01. Brain Wellness and Aging

Support: T32AG061892
NIH Grant RF1AG064914

Title: Elucidating the role of tau in cortical neuronal networks

Authors: *B. S. ULM[1,3,4], C. HANSEN[2], S. VELASCO[2], W. HACHMEISTER[2], M. BU[1,3,4], B. I. GIASSON[1,3,4], J. M. LEWIS[1,3,4], H. KHOSHBOUEI[1,3];

[1]Neurosci., [2]Univ. of Florida, Gainesville, FL; [3]McKnight Brain Inst., Gainesville, FL; [4]Ctr. for Translational Res. in Neurodegenerative Dis., Gainesville, FL

Abstract: *Rationale and Objective:* Tau is one of the most prevalent proteins found in protein aggregates of Alzheimer's disease and certain frontotemporal dementias. Despite this, the connection between tau regulation of neuronal activity and neurodegenerative decline is less understood. The objective of this study was to determine the influence of tau dysregulation on neuronal activity and functional connectivity, at baseline and following pharmacologic modulation of neuronal activity.
Methods: Murine primary cortical cultures were co-transduced with custom split vector mCherry-expressing AAV with or without 0N4R human tau (WT or P301L mutant) and GCaMP6f (a fluorescent calcium indicator). Live-cell imaging was used to quantify the spontaneous neuronal activity, as measured by increase in fluorescence intensity of GCaMP6f, at baseline and following inhibition of $GABA_A$ R (10μM) or NMDAR antagonism (20μM). The data were analyzed via manual segmentation and custom coded analysis to determine features of individual neuronal activity, such as calcium spike rates, inter-spike interval coefficient of variation, and mean increase in intensity (from the minima). In addition, we employed graph theory-based analysis of neuronal networks to determine changes in functional connectivity; these include normalized node degree, degree assortativity, and clustering coefficient. To determine tau-induced pathological progression, the cortical neuronal cultures were assessed at three time-points: 10 days, 15 days, and 20 days after tau (or control AAV) transduction (n=12-14 per condition). To determine whether or not effects of tau requires fibrillization, we also investigated the effect of tau fibril administration on neuronal activity and neuronal network features.
Results: While preliminary, our results thus far suggest that increased 0N4R human tau expression in cortical neurons does not substantially change individual neuronal activity or functional neuronal network structure. These results indicate that tau regulation of functional connectivity in early stages of tau overexpression in cortical neurons is negligible, below our detection limit, or importantly is limited by the model (overexpression of tau as an incomplete facsimile of primary tauopathy). Ongoing studies investigate the effects of tau fibrils on cortical neuronal activity and functional connectivity to model late-stage tau pathology.

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Poster

P184. Molecular Mechanisms: Brain Aging, Glia and Neuroinflammation

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P184.04

Topic: C.01. Brain Wellness and Aging

Title: Reduced expression of the cell intrinsic clock protein Bmal1 in myeloid cells accelerates cognitive decline and alters microglial function in aging mice

Authors: *C. AGBAEGBU IWEKA, E. BLACHER, C. TSAI, Q. WANG, K. ANDREASSON;
Stanford Univ., Stanford, CA

Abstract: The circadian clock plays a fundamental role in the temporal coordination of the external environment with internal physiological processes. These processes include regulation of the sleep/wake cycle, feeding behavior, core body temperature, and immune responses. With aging, the ability to rhythmically regulate these processes progressively declines. Aging is associated with development of chronic inflammation that is linked to multiple age-associated diseases, including cognitive decline. Several studies have demonstrated that tissue-specific deletion of Bmal1, a transcription factor and regulator of circadian rhythms, can accelerate organ-specific aging, for example the development of sarcopenia in muscle, bone calcification in bone and cataracts in the eye. Here, we show that in myeloid cells, conditional knockout of Bmal1 increases systemic inflammation and accelerates cognitive decline in aging mice. In the brain, we found increased numbers of microglia displaying less morphological complexity and reduced levels of CD68 in microglia, suggesting that loss of Bmal1 in microglia disrupts lysosomal function. Moreover, myeloid Bmal1 deletion led to significant increases in pre- and post-synaptic proteins in aged mice, as well as decreased levels of C1q, which is required for synaptic pruning. Thus, loss of circadian regulation in brain microglia accelerates age-associated cognitive decline by a mechanism that may involve a deficit in synapse removal.

Disclosures: C. Agbaegbu Iweka: None. **E. Blacher:** None. **C. Tsai:** None. **Q. Wang:** None. **K. Andreasson:** None.

Poster

P184. Molecular Mechanisms: Brain Aging, Glia and Neuroinflammation

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Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P184.05

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant R01 NS115809

Title: Aging alters spontaneous astrocytic endfoot calcium signals within the dorsolateral striatum of mouse brain slices

Authors: S. M. ZARATE[1], T. E. HUNTINGTON[2], P. BAGHER[4], *R. SRINIVASAN[3];
[1]Neurosci. and Exptl. Therapeut., Texas A&M Hlth. Sci. Ctr., Bryan, TX; [2]Neurosci. and Exptl.

Therapeut., Texas A&M Univ., Bryan, TX; [3]Neurosci. and Exptl. Therapeut., Texas A&M Univ., College Station, TX; [4]Med. Physiol., Texas A&M Univ. Hlth. Sci. Ctr., Bryan, TX

Abstract: Emerging evidence suggests that aging-related dysfunction in the neurovascular unit (NVU) can potentially trigger chronic neurodegenerative processes such as Alzheimer's and Parkinson's disease. Among the brain regions prone to neurodegeneration, the striatum is a particularly vulnerable brain region, demonstrating a high percentage of lacunar infarcts, the accumulation of toxic protein aggregates, and an increased propensity for the loss of blood brain barrier (BBB) integrity. Since astrocytic endfeet are thought to regulate NVU function via spontaneous Ca^{2+} signals, we asked if aging is associated with specific changes in spontaneous Ca^{2+} signals within astrocytic endfeet from live brain slices of the dorsolateral striatum (DLS). The DLS of young (2-month-old) and aged (24-month-old) mice were stereotaxically injected with an adeno associated virus (AAV) expressing the membrane tagged genetically encoded Ca^{2+} indicator, lck-GCaMP6f, driven by an astrocyte specific GfaABC1D promoter (AAV-lck-GCaMP6f). Prior to obtaining DLS slices, mice were transcardically perfused with tomato-lectin in order to visualize capillaries within the DLS. Spontaneous endfoot Ca^{2+} events surrounding capillaries were imaged in DLS brain slices from young and aged mice using confocal microscopy. We found that in aged mice, endfoot Ca^{2+} events were 77% larger with a 50% increase in event half width. Additionally, we found that endfoot Ca^{2+} waves in aged mice displayed a 2-fold greater velocity than young mice. Sequential depletion of extracellular Ca^{2+} followed by endoplasmic reticulum (ER) Ca^{2+} stores revealed an increase in the dependency on ER Ca^{2+} only in aged mice. Because the ER is a major Ca^{2+} source for mitochondria, we utilized GCaMP6f directed to astrocytic mitochondria (AAV-mito-GCaMP6f) to record mitochondrial Ca^{2+} events in endfeet. These experiments revealed a 50% increase in the frequency of spontaneous Ca^{2+} events in aged astrocytic endfoot mitochondria. In addition, we found a significant 2-fold reduction in the major ER Ca^{2+} buffering protein, calreticulin in aged mice. Together, these data suggest that astrocytic endfeet in aged mice display altered ER Ca^{2+} and Ca^{2+} buffering mechanisms that could potentially contribute to NVU dysfunction and a loss of BBB integrity.

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Poster

P184. Molecular Mechanisms: Brain Aging, Glia and Neuroinflammation

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Program #/Poster #: P184.06

Topic: C.01. Brain Wellness and Aging

Support: NIH grant F31NS116947

Hydrocephalus Association 2020 Innovator Award
Fraternal Order of Eagles (University of Iowa)

Title: Kaolin mouse model of normal pressure hydrocephalus

Authors: *M. TISH, J. C. GEERLING;
Neurol., Univ. of Iowa, Iowa City, IA

Abstract: Normal pressure hydrocephalus (NPH) is a chronic form of communicating hydrocephalus in older adults, in which cerebral ventricles expand due to poor absorption of cerebrospinal fluid (CSF). NPH is often idiopathic but may develop following subarachnoid hemorrhage, meningitis, or other cerebral processes that impair CSF circulation. NPH produces a triad of progressive symptoms: gait impairment, urinary incontinence, and cognitive dysfunction. A mouse model for NPH would help accelerate neuroscience research into the mechanisms of these symptoms. Kaolin has been used to induce hydrocephalus in cats and rodents for decades, but usually produces a rapidly progressing lethal hydrocephalus. Additionally, the complete triad of symptoms in human NPH has not been reported on following kaolin induced hydrocephalus. We developed a chronic, survivable model of communicating hydrocephalus with symptoms similar to human NPH. Injecting 5 µL of a 10-15% kaolin (aluminum silicate) suspension into the cisterna magna of adult mice produces gradually progressive enlargement of the cerebral ventricles. We use brain MR imaging to measure the cerebral ventricular volume every two weeks through week 10 post-surgery, along with weekly tests of bladder function (Micturition Video Thermography), gait and balance, plus a variety of behavioral testing before and 10 weeks after surgery. Hydrocephalic mice, but not saline-injected controls, develop impairments in gait and balance and urinary frequency with incontinent features. Intracranial pressure is not elevated at any time points measured (3 days, 1, 2, 8-12 weeks). Ventricular enlargement is correlated with weight loss in the two weeks following kaolin injection. Brain tissue from these mice shows several abnormalities near the ventricles that may help account for differential symptoms. This mouse model allows us to investigate the pathophysiology of chronic hydrocephalus, specifically including mechanisms that link ventricular enlargement in NPH to disabling gait and bladder symptoms.

Disclosures: M. Tish: None. **J.C. Geerling:** None.

Poster

P184. Molecular Mechanisms: Brain Aging, Glia and Neuroinflammation

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P184.07

Topic: C.01. Brain Wellness and Aging

Support: IOER funds

Title: Exercise protects against inflammation by modulating rat hippocampal NF-kB and nucleus accumbens FosB/ΔFosB

Authors: *C.-M. LEE[1], L. POINTS[1], K. ALGANEM[2], B. POPE[1], R. MCCULLUMSMITH[2], L.-L. YUAN[1];
[1]Dept. of Physiol. and Pharmacol., Des Moines Univ., Des Moines, IA; [2]Dept. of Neurosci., Univ. of Toledo, Toledo, OH

Abstract: It is well-established that exercise exerts beneficial effects on the central nervous system, such as protecting against neuronal aging and inducing stress-resilience. However, significant individual differences exist in exercise motivation, and the underlying mechanisms linking exercise to brain health remain unclear. Using rat voluntary wheel running, we modeled for acute exercise: rats were allowed to run during a 5-hour session after 4 days of training and subsequent 2 days of rest. They were then grouped based on running distances. With high throughput active kinome profiling on rat hippocampus, we discovered exercise significantly reduces the activity of several kinases in the NF-kB activation pathway responsible for cellular inflammation, including COT, IKK, PKD, and RIPK. Subsequent validation with transcriptional analysis showed that with an acute bout of running, NF-kB activity in the hippocampus is significantly repressed compared to sedentary controls, especially in high-running rats. Additionally, we found that acute exercise induces FosB/ ΔFosB expression in the nucleus accumbens, which drives motivation in reinforced behaviors. This is intriguing because while FosB/ΔFosB canonically responds to repeated and chronic exposure to certain antidepressants or drugs of abuse, here we show that a single period of exercise is sufficient to induce its expression. In summary, we propose that acute exercise may exert anti-inflammatory effects via downregulation of NF-kB pathway in the hippocampus, and the motivation underlying exercise may be related to FosB/ΔFosB expression in the nucleus accumbens. Future directions include explicating individual differences in exercise motivation and examining other brain regions.

Disclosures: C. Lee: None. **L. Points:** None. **K. Alganem:** None. **B. Pope:** None. **R. Mccullumsmith:** None. **L. Yuan:** None.

Poster

P184. Molecular Mechanisms: Brain Aging, Glia and Neuroinflammation

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P184.08

Topic: C.01. Brain Wellness and Aging

Title: Major changes to endothelial cells and astrocytes following inhibition of a detrimental age-associated enzyme

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Abstract: Cognitive impairment is a major consequence of aging and is associated with many aging-related diseases. We have identified a detrimental enzyme, leukotriene A4 hydrolase (LTA4H), that is increased in the plasma of humans with age, and high circulating levels of this enzyme are correlated with cognitive impairment in an Alzheimer's patient population. We found that dosing mice with recombinant enzyme at concentrations found in aged human plasma drives cognitive impairment in young mice indicating that it can act as a detrimental factor. Furthermore, inhibition of enzyme activity in aged mice improves cognitive decline and blood brain barrier permeability. Using unbiased single cell RNA sequencing, we identified changes in brain endothelial cells and astrocytes that may contribute to these improvements. Taken together these results identify a novel aging-related pathway that may be targeted to treat age-related cognitive decline.

Disclosures: M.K. Campbell: A. Employment/Salary (full or part-time):; Alkahest. **J.M. Adams:** A. Employment/Salary (full or part-time):; Alkahest. **S. Rege:** A. Employment/Salary (full or part-time):; Alkahest. **A.T. Liu:** A. Employment/Salary (full or part-time):; Coda Biotherapeutics Inc. **R. Britton:** A. Employment/Salary (full or part-time):; Alkahest. **A.L. Nguyen:** A. Employment/Salary (full or part-time):; Alkahest. **N.V. Vu:** A. Employment/Salary (full or part-time):; Alkahest. **D.Y. Kirsher:** A. Employment/Salary (full or part-time):; Alkahest. **D.P. Leone:** A. Employment/Salary (full or part-time):; Alkahest. **R. Harish:** A. Employment/Salary (full or part-time):; Alkahest. **B. Szoke:** A. Employment/Salary (full or part-time):; Alkahest. **S.S. Minami:** A. Employment/Salary (full or part-time):; Alkahest. **E. Czirr:** A. Employment/Salary (full or part-time):; Alkahest.

Poster

P184. Molecular Mechanisms: Brain Aging, Glia and Neuroinflammation

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P184.09

Topic: C.01. Brain Wellness and Aging

Support: NIH Grant RF1AG028271

Title: Tlr4 antagonism ameliorates persistent post-operative cognitive dysfunction

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Abstract: Post-operative cognitive dysfunction (POCD) is a complication experienced by many surgical patients. POCD is characterized by cognitive impairments ranging from mild confusion to difficulty with executive functions to inability to form long-term memories to dementia. Persistent POCD, lasting weeks to months, is most commonly experienced by older individuals and is associated with an increased vulnerability to developing Alzheimer's Disease. Our previous work demonstrated that, in older rats, surgery alone evoked a neuroinflammatory response and hippocampal-dependent long-term memory dysfunction lasting 4 days. Treating the rats with morphine, often administered post-operatively for pain management, exaggerated and prolonged this surgery-induced inflammatory response, leading to persistent POCD lasting at least 8 weeks. Here, we explored the role of toll-like receptor 4 (TLR4) in this process. TLR4 is an innate immune pattern recognition receptor and is expressed by microglia. Importantly, activation of TLR4 initiates a proinflammatory cytokine cascade. With age, microglia naturally become sensitized, exhibiting greater expression of TLR4 and increasing the likelihood of exaggerated neuroimmune responses following a challenge. Of note, exaggerated neuroinflammation potently inhibits synaptic plasticity necessary for long-term memory. Interestingly, morphine has recently been shown to have an inflammatory role via TLR4 activation. We hypothesized that surgery, aging, and morphine synergize via TLR4 activation to cause persistent POCD. To test this, we subjected 24 month old male rats to laparotomy as a model of POCD and administered analgesic morphine for 1 week. Two experiments were performed: the TLR4-specific antagonist lipopolysaccharide from the bacterium *Rhodobacter sphaeroides* (LPS-RS) was centrally administered either 1) 1x immediately before surgery or 2) 1x/d x7d beginning the day after the last morphine injection post-operatively. In both experiments, hippocampal-dependent long-term memory was assessed 4 weeks after surgery using a contextual fear conditioning paradigm. Both pre- and post-operative treatment with the TLR4 antagonist prevented the persistent POCD memory impairment. These results indicate that TLR4 plays a mediating role in the development of persistent POCD, although the specific mechanisms of this process remain to be elucidated. These data also suggest that blocking central TLR4 activation may be a promising therapeutic to treat POCD.

Disclosures: S.M. Muscat: None. **N.P. Deems:** None. **K.C. Rice:** None. **S.F. Maier:** None. **R.M. Barrientos:** None.

Poster

P184. Molecular Mechanisms: Brain Aging, Glia and Neuroinflammation

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Program #/Poster #: P184.10

Topic: C.01. Brain Wellness and Aging

Support: R01 AG051902

Title: Astrocyte immunosenescence in IL-10 and cholesterol signaling in the aged brain impairs microglial regulation following peripheral immune activation.

Authors: *S. M. O'NEIL, E. E. HANS, S. JIANG, L. M. WANGLER, J. P. GODBOUT; Dept. of Neurosci., The Ohio State Univ., Columbus, OH

Abstract: Microglia, the innate immune cells of the CNS, develop a pro-inflammatory, "primed" profile with age. Using single-cell RNA-sequencing (scRNA-Seq), we confirmed microglia in the brain of aged mice (18 months old) had an amplified (4 h) and prolonged (24 h) neuroinflammatory response to peripheral lipopolysaccharide (LPS) challenge compared to adults (3 months old). Overall, we observed several age-, LPS-, and time-dependent differences in the scRNA-Seq subclusters of microglia. Ingenuity Pathway Analysis of upstream regulators and canonical pathways revealed impaired regulation of an activated, neuroinflammatory state within microglia. In parallel, astrocytes in the aged brain exhibited a profile consistent with "immunesenescence" both 4 and 24 h after peripheral LPS challenge. For example, aged astrocytes showed reduced anti-inflammatory signaling and cholesterol biosynthesis, two pathways by which astrocytes regulate the inflammatory profile of microglia. One of the pathways reduced in the aged brain was interleukin 10 receptor (IL-10R) signaling. This pathway increases astrocytic expression of transforming growth factor beta (TGFβ), an anti-inflammatory cytokine with abundant receptor expression on microglia. Therefore, transgenic astrocytic *Il10ra*^{KO} mice were generated to determine if impaired IL-10R/TGFβ signaling within astrocytes is sufficient to cause a dysfunctional neuroinflammatory response in microglia. Indeed, astrocytic *Il10ra*^{KO} induced exaggerated sickness behavior and a prolonged neuroinflammatory response to peripheral LPS challenge, including increased social avoidance with amplified *Il1b* and *Tnf* mRNA expression in microglia. In summary, these novel data show astrocytes display an immunosenescent profile with age and, in response to peripheral LPS challenge, exhibit IL-10R signaling deficits and a lack of cholesterol biosynthesis, both leading to the inability to resolve microglia activation.

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Poster

P184. Molecular Mechanisms: Brain Aging, Glia and Neuroinflammation

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Program #/Poster #: P184.11

Topic: C.01. Brain Wellness and Aging

Support: R01MH101130
R01MH113892
R01AG061200

Title: Aging triggers an upregulation of a multitude of cytokines in the male and especially the female rodent hippocampus but more discrete changes in other brain regions

Authors: N. GORNY[1], L. PORCHER[1], S. BRUCKMEIER[1], S. D. BURBANO[2], J. FINNELL[2], J. KLETT[2], S. K. WOOD[2], *M. KELLY[1];
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Abstract: Background: Despite widespread acceptance that neuroinflammation contributes to age-related cognitive decline, studies comparing protein expression of cytokines in young versus old brains are surprisingly limited in terms of the number of cytokines and brain regions studied. Complicating matters, discrepancies abound—particularly for interleukin 6 (IL-6)—possibly due to differences in sex, species/strain, and/or the brain regions studied. Methods: As such, we clarified how cytokine expression changes with age by using a Bioplex and Western blots to measure multiple cytokines across several brain regions of both sexes, using 2 mouse strains bred in-house as well as rats obtained from NIA. Parametric and nonparametric statistical tests were used as appropriate. Results: In ventral hippocampus of C57BL/6J mice, we find age-related increases in IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-6, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-17, eotaxin, G-CSF, interfeuron δ, KC, MIP-1a, MIP-1b, rantes, and TNFα that are generally more pronounced in females, but no age-related change in IL-5, MCP-1, or GM-CSF. We also find aging is uniquely associated with the emergence of a module (a.k.a. network) of 11 strongly intercorrelated cytokines, as well as an age-related shift from glycosylated to unglycosylated isoforms of IL-10 and IL-1β in ventral hippocampus. Interestingly, age-related increases in extra-hippocampal cytokine expression are more discreet, with prefrontal cortex, striatum and cerebellum of male and female C57BL/6J mice demonstrating robust age-related increase in IL-6 expression but not IL-1β. Importantly, we found this widespread age-related increase in IL-6 also occurs in BALB/cJ mice and Brown Norway rats, demonstrating conservation across species and rearing environments. Conclusions: Thus, age-related increases in cytokines are more pronounced in the hippocampus compared to other brain regions and can be more pronounced in females versus males depending on the brain region, genetic background, and cytokine examined.

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Poster

P184. Molecular Mechanisms: Brain Aging, Glia and Neuroinflammation

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P184.12

Topic: C.01. Brain Wellness and Aging

Support: RF1AG068292, G-quadruplex DNA in senescence of the neurovascular unit

Title: Evidence for nucleolar G-quadruplex-DNA formation in driving genomic instability in neurons

Authors: *R. DIAZ ESCARCEGA[1], N. TABOR[1], J. F. MORUNO MANCHON[1], A. URAYAMA[1], S. P. MARRELLI[1], L. D. MCCULLOUGH[1], A. S. TSVETKOV[1,2];
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Abstract: Non-canonical base pairing between four guanines (G) within single-stranded G-rich sequences and self-stacking of G-quartets result in the formation of a columnar four-stranded DNA structure-the G quadruplex (G4 or G4-DNA). G4-DNA regulates a wild variety of DNA-dependent processes including transcription, replication, and telomere function. Overly stabilized G4-DNA may form at a nucleosome-depleted, transcriptionally active site, acting as a physical barrier for a DNA polymerase and eventually leading to DNA damage. Genomic instability contributes to neurodegenerative disorders, but potential G4-DNA associated mechanisms of genomic instability in neurons are not understood. Using cultured rat primary neurons, we found that the neuronal nucleolus is enriched in G4-DNA. Pharmacologically stabilizing G4-DNA leads to DNA damage primarily in the nucleolus. We conclude that nucleolar G4-DNA may be an important driver of genomic instability, contributing to neuronal aging and neurodegeneration.

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Poster

P192. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches I

Location: Virtual Only

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Program #/Poster #: P192.01

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

Support: 5 T32 GM 7752-42
NIH R01AG065541

Title: Human brain-derived extracellular vesicles contain full-length mRNAs

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Abstract: Exosomes are 30-150nm extracellular vesicles (EVs) released by fusion of the multivesicular body, a type of late endosome, with the plasma membrane. Exosomes contain nucleic acids and proteins and are thought to be a means of intercellular communication. Within the brain, exosomes are known to be released upon neuronal depolarization, and to be exchanged between both neurons and glia. The protein contents of brain-derived exosomes have garnered interest in the field of neurodegeneration, for example as conduits for transmission of misfolded proteins. However, transcriptomic RNAs in EVs have been less studied. We hypothesize that neural cells may communicate intercellularly through the exchange of mRNAs transported in EVs. We isolated bulk and EV mRNA from human post-mortem frontal cortex of 24 brains: 12 from donors with Alzheimer's Disease and 12 that were neurologically non-diseased. Short-read sequencing revealed EV enrichment of transcripts relating to oxidative phosphorylation and mitochondrial function. Long-read sequencing was used to identify transcripts that could potentially be functional upon translation, revealing the presence of diverse full-length transcripts. We also identified novel RNA isoforms that contained structures such as intra-exonic junctions (IEJs), previously associated with somatic gene recombination. Our data suggest that brain-derived EVs can transfer full-length mRNAs between and amongst cells of the brain, expanding the transcriptome of neural cells.

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Poster

P192. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P192.02

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

Support: NIGMS T32 GM008541
NIH/NIA R21AG056947
NIH/NIA R21 AG061553-01

Title: A Prodromal View of the TgF344-AD Rat Model of Alzheimer's Disease Using Single Nuclei RNA sequencing.

Authors: *A. TIPTON[1], J. GEORGE[2], V. KUMARESAN[3], M. RATNER[2], D. H. FARB[4], S. J. RUSSEK[5];
[2]Dept. of Exptl. Therapeut., [3]Lab. of Mol. Neurobiology, Dept. of Pharmacol., [1]Boston Univ. Sch. of Med., Boston, MA; [5]Pharmacol. and Grad Prog for Neurosci., [4]Boston Univ. Sch. Med., Boston, MA

Abstract: Alzheimer's disease (AD) is a devastating disorder characterized by gradual decline in memory and cognitive functioning, leading to eventual loss of independence and significant caregiver burden and healthcare costs at a societal level. Despite extensive investment into development of AD treatments, no disease modifying drugs are available. Recent failures of phase 3 clinical trials for mAb's targeting Aβ plaques have led to renewed interest in early signatures of disease in hopes of altering disease trajectory through early intervention. Key to such efforts is capturing a molecular window into prodromal stages. The TgF344-AD rat shows overt pathology (including Aβ plaques, frank neuronal loss, and endogenous tau pathology) at 16 and 26 mo, but not at 6 mo (Towne, 2013). However, variable behavioral phenotypes in 6 mo Tg rats, including heightened anxiety and impaired spatial reversal learning, have been reported (Pentkowski, 2018; Rorabaugh, 2017). Our recent discoveries (doi: 10.1101/2021.05.10.443512), using *in vivo* recordings during awake immobility, have uncovered an early dysfunction in the transgenic tri-synaptic hippocampal circuit not previously appreciated. To determine its molecular basis, we used 10x Genomics' v3 gene expression assay to perform snRNA-seq on freshly dissected hippocampi from 6 mo male and female littermate pairs of Tg and Wt rats (n=16). ~2000 cells/subject were collected, and cDNA libraries were sequenced (NovaSeq 600) to a depth of ~120k reads/nuclei. Cell Ranger Pipeline demultiplexed and aligned reads to rat pre-mRNA reference and generated gene count matrices. QC metrics were used to filter out dying cells, empty wells, and likely multiplets, resulting in a final dataset of 33,079 high quality nuclei. Nuclei were clustered (Louvain graph-based) and identity determined with cell-type specific markers curated from the literature. Identity was collapsed into broad neuronal and glial cell types for differential gene expression (DEG) across genotype (Hurdle Model). DEGs revealed profound alterations between Tg and Wt in oligos, dentate granule cells (DGCs), and excitatory neurons (ExcNs). (FDR cutoff, 0.05 and 1111 (DGCs) and 1070 (ExcN's) DEGs in Tg vs Wt.) Pathway enrichment revealed dysregulation relevant to memory function: including decreased synaptogenesis, neurite branching, proliferation, and microtubule and cytoskeleton dynamics. DEG's were enriched for neurological signs including epilepsy, seizures, hyperactivity, and dementia. Validation efforts are ongoing via multiplexed ISH and RTqPCR of sorted nuclei populations. Future work includes earlier timepoints and network rescue experiments.

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Poster

P192. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches I

Location: Virtual Only

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Program #/Poster #: P192.03

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

Support: U24AG061340
RF1AG057443
U54AG065187

Title: Ad knowledge portal and agora: resources that empower the research community to find, access, and re-use data and tools related to dementia and aging

Authors: *J. SCHNEIDER, A. VANDER LINDEN, M. FAZZA, J. GOCKLEY, A. K. GREENWOOD, D. HAN, L. HEATH, N. KAUER, K. LEAL, Z. J. LEANZA, K. MONTGOMERY, L. OMBERG, N. PERUMAL, P. PUGALENTHI, M. A. PETERS, W. POEHLMAN, S. K. SIEBERTS, C. SUVER, T. THYER, R. H. YAXLEY, J. WILEY, L. MANGRAVITE;
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Abstract: To identify disease modifying therapies and drug targets for Alzheimer’s disease (AD), it is necessary to assess the impact of cellular and molecular dysfunction on disease etiology. The AD Knowledge Portal (Portal) (https://adknowledgeportal.org) and Agora (https://agora.adknowledgeportal.org) are community-driven resources that have been developed to support researchers as they: 1) identify new molecular hypotheses and mechanisms, 2) evaluate hypotheses via independent experimental assessments, and 3) prioritize new molecular mechanisms for therapeutic development. The Portal and Agora are free, open access tools built to maximize therapeutic discovery by enabling researchers to re-use data from 41,000+ biospecimens collected from 12,000+ individuals across four species, as well as access analytical results related to putative drug targets. The Portal and Agora empower the research community with a trusted repository of data, computational and experimental tools and potential gene targets to effectively and efficiently test target hypotheses and therapeutics.
The Portal was created to support research outputs from the National Institute on Aging funded Accelerating Medicines Partnership-Alzheimer’s Disease, and has expanded to support 55 grants generating data and computational/experimental tools related to dementia and aging. Generated from central and peripheral measurements from brain banks, longitudinal cohorts, and multiple model systems, available data spans 55+ assays ranging from genomic variants to metabolomics, imaging, cognitive assessments, and more. Model systems represented in the Portal include iPSC-derived cell types and organoids, and novel Late Onset AD mouse models based on gene targets identified from human data. These mouse models are available with no limitation on use. Most data types in the Portal are available in both raw and processed formats. The Portal also features a cloud-based analytical workspace providing access to preconfigured computational

resources to process, integrate, and analyze data. A subset of the processed data is also presented in Agora, a visual results explorer that compiles evidence for the association of genes with AD. Agora hosts a list of nominated targets and presents results of 'omics analyses generated from Portal data, and now, also includes a catalogue of details and results from targeted validation studies. The AD Knowledge Portal and Agora offer the research community an accessible, rich source of data, tools, and results.

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Poster

P192. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches I

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

Support: NIH Grant R01AG046170
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Title: A systematic functional investigation of CNVs in AD based on omics data integration

Authors: *C. MING[1], M. WANG[1], Q. WANG[1], R. NEFF[1], E. WANG[1], Q. SHEN[1], J. S. REDDY[3], X. WANG[3], M. ALLEN[4], N. ERTEKIN-TANER[4], P. L. DE JAGER[5], D. A. BENNETT[6], V. HAROUTUNIAN[2], E. SCHADT[1], B. ZHANG[1];
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Abstract: The pathogenic mechanism of copy number variations (CNVs) in late-onset Alzheimer's disease (LOAD) lacks genome-wide systematically study, even though CNVs have been found to play a significant role in many neurological diseases such as Parkinson's disease, schizophrenia, and mental retardation. A systematic functional study of CNVs through omics-

levels can provide valuable insights into the mechanism of LOAD. In this study, we employed four complementary CNV calling approaches to comprehensively identify CNVs in the whole genome sequencing data from 1,603 North American Caucasian individuals across three LOAD cohorts including the Mount Sinai/JJ Peters VA Medical Center Brain Bank (MSBB) AD cohort, the ROSMAP cohort, and the Mayo Clinic cohort. Integrative analysis of the identified CNVs and the matched transcriptomic, clinical, and pathological data was then performed to determine the functional impacts of these CNVs.By comparing 701 LOAD cases with 710 non-AD cases, we identified 3,012 rare AD-specific CNVs which are enriched for transcriptional regions involved in biological processes such as cellular glucuronidation, neuron projection, and multicellular organismal signaling. By further integrating clinical, pathophysiological, and transcriptomic data, we found that common CNVs affect the transcription levels of genes involved in MHC Class II receptor activity and interferon-gamma mediated signaling across seven brain regions, supporting previous reports of the increased immune response in AD. Three CNVs are significantly negatively correlated with the Braak score in the DLPFC region. CNV-centered correlation networks integrating matched multi-omics and clinicopathological data pinpoint one novel CNV, a key regulator for immune response, and further provide many novel gene targets which connect CNVs and clinical and pathological traits of AD. All consensus CNVs of the three cohorts have been uploaded to the UCSC genome browser track (http://genome.ucsc.edu/s/c6ming2/AMPAD.CNVs).In summary, we systematically identified AD-specific CNVs and further established their functional contexts using CNV-gene correlation networks. These findings deepen our understanding of genetic mechanisms underlying AD.

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Poster

P192. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P192.05

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

Title: Association of Polygenic Risk Scores with Age-at-Onset and Rate of Cognitive Decline in Alzheimer's Disease

Authors: D. CHRISTY[1], E. GUSTAVSSON[3], D. EVANS[2], G.-Y. HSIUNG[2], M. J. FARRER[4], ***H. B. NYGAARD**[2];
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Abstract: Background: Alzheimer's disease (AD) is a complex and heterogeneous neurodegenerative condition for which genetic susceptibility is one of the most important risk factors. Genome-wide association studies (GWAS) have identified 32 disease associated genomic loci, each with limited clinical applicability due to small effect size. Polygenic risk scores (PRS) provide an opportunity to assess the cumulative association by combining the effect of each susceptibility loci into a single value. Methods:We genotyped 50-disease associated single nucleotide polymorphisms (SNPs) in 227 patients clinically diagnosed with AD for which we had longitudinal clinical information. We assessed the cumulative association of the SNP set to age-at-onset (N=227) and rate of cognitive decline as defined by the linear regression of Mini-Mental State Examination (MMSE) scores (N=176) through use of two PRS. Moreover, due to *APOE*'s large effect size we investigated it individually for association to age-at-onset. Results: The polygenic risk scores form a significant inverse association with age-at-onset ($r= -0.36$, $p<0.0001$) and positive association with annualized MMSE rate of decline ($r=0.56$, $p<0.0001$). We additionally see a significant positive association between age-at-onset and annualized MMSE decline ($r=0.33$, $p<0.0001$). Lastly, we observe APOE ε4 allele dose dependent effects which lower age-at-onset dependent on the number ε4 alleles a subject possesses. Conclusions: These results suggest recent GWAS disease associated SNPs not only associate with disease but with other phenotypic attributes.

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Poster

P192. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches I

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

Support: NIH Grant 1RF1AG071566-01
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ADNI: DOD Grant W81XWH-12-2-0012
Cleveland Clinic Keep Memory Alive Young Investigator Award

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a private grant from Peter and Angela Dal Pezzo

Title: Associations between polygenic hazard score (PHS) and Alzheimer's Disease (AD) progression and biomarkers following the stratifying by ApoE genotypes and sex

Authors: *X. ZHUANG[1], R. TILLETT[2], R. YOUNG[2], D. CORDES[3], E. OH[2];
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Abstract: Introduction. Polygenic hazard scores (PHS), derived by integrating AD-associated SNPs into a cox regression model, reveals age-associated AD risks. Previous studies have reported various associations between PHS and AD progressions and biomarkers. However, the additional effects of PHS beyond ApoE genotypes remain unclear since different ways of adjusting ApoE led to various conclusions. Moreover, a sex-dependent autosomal effect has been reported on AD progression clinically, but whether sex will moderate the above associations remains unclear. In this study, using data from Alzheimer's Disease Neuroimaging Initiatives, we examined the association between PHS and AD progression (stable/progressive mild cognitive impairment (MCI)) and biomarker (PET-amyloid (Ab)) after stratifying by ApoE genotypes. We further examined whether these associations were moderated by sex. **Methods.** 703 subjects with an initial clinical diagnosis (DX) of MCI and follow-up visits were included, with 306 MCI subjects progressed to AD (MCI-p) longitudinally and 397 MCI subjects remained stable (MCI-s). 297 MCI-s subjects and 133 MCI-p subjects went through AV-45 PET imaging while maintaining a clinical MCI DX. Subjects' demographics, clinical DX, ApoE genotype, PHS, and whole brain Ab standardized uptake value ratio (SUVR) at every visit were obtained. PHS was first compared between MCI-s and MCI-p groups, with sex as the 2nd factor in the ANOVA analysis. Linear regression was used to determine whether there was an association (1) between PHS and MCI to AD progression time in MCI-p subjects; and (2) between PHS and Ab level in all MCI subjects. All regressions analyses were repeated with sex as a moderator, and within each ApoE genotype. **Results.** *PHS and AD progression.* MCI-p subjects share significantly higher PHS (p<0.001) than MCI-s subjects. Only E3E3 MCI-p subjects have marginally higher PHS than E3E3 MCI-s subjects (p=0.07). In MCI-p subjects, higher PHS is significantly associated with shorter MCI to AD progression time (p<0.001). This association holds only in E3E4 MCI-p subjects (p=0.01). *PHS and AD biomarkers.* PHS is significantly associated with whole brain Ab level in all MCI subjects (p<0.001). This association significantly differs by sex (p=0.03) but does not differ between MCI-p and MCI-s subjects. This association further holds significant for only E3E4 MCI subjects (p=0.05) and is mostly driven by E3E4 MCI women (p=0.04). **Conclusion.** PHS is significantly associated with AD progression. Non-ApoE SNPs contribute to clinical conversion times from MCI to AD. PHS is also significantly associated with whole brain Ab burden, and this association differs by sex.

Disclosures: X. Zhuang: None. **R. Tillett:** None. **R. Young:** None. **D. Cordes:** None. **E. Oh:** None.

Poster

P192. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P192.07

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

Support: NIH U01AG061357
NIH 3U01AG061357-02S1
NIH 5P30AG066511-02
NIH P30NS055077
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Title: Resolving racial differences in the brain proteome of African Americans with Alzheimer's disease

Authors: *E. MODESTE, L. PING, D. M. DUONG, M. GEARING, J. J. LAH, A. I. LEVEY, N. T. SEYFRIED;
Emory Univ., Atlanta, GA

Abstract: Chronic health conditions including cardiovascular disease and diabetes, lower quality and level of education, higher rates of poverty, and greater exposure to discrimination disproportionally affect African Americans, putting this population at a heightened risk for Alzheimer's disease (AD) and related dementias. Neuropathological hallmarks of AD include accumulations of amyloid-beta (Aβ) plaques and tangles of hyperphosphorylated tau in the brain. Levels of Aβ and tau in cerebrospinal fluid (CSF) can act as proxies for AD pathology in the brain, thus providing the unique ability to monitor AD in living patients. Emerging biomarker studies in CSF show that African Americans have lower levels of tau, compared to Caucasians. This suggests that race may influence tau and related AD pathophysiology in African Americans. Whether race impacts tau levels or other pathologies in brain is not well established. Here, we sought to characterize the brain proteome of Caucasians and African Americans with AD. Using multiplexed mass spectrometry-based proteomics coupled with off-line fractionation, we quantified ~8,000 proteins across 129 matched African-American and Caucasian AD and control postmortem brain tissues. Interestingly, we were able to stratify cases by their self-identified race using a subset of race-specific proteins identified by variance partition. Notably, characteristic hallmarks of AD, including increases in Aβ peptide and total tau levels, did not differ by race. Following phosphopeptide enrichment using iron-chelated magnetic beads, greater than 60 tau phosphopeptides were identified and quantified, but none showed statistically significant differences between African Americans and Caucasians with AD. Co-expression network analysis, however, did reveal pathways reflective of ribosomal function, vascular biology, and RNA splicing that differed between African Americans and Caucasians in AD. Together, this data suggests that race is involved in the manifestation of AD beyond, the pathological hallmark proteins, Aβ and tau.

Disclosures: E. Modeste: None. **L. Ping:** None. **D.M. Duong:** None. **M. Gearing:** None. **J.J. Lah:** None. **A.I. Levey:** None. **N.T. Seyfried:** None.

Poster

P192. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P192.08

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

Support: Stanley Center
Alzheimer's Association
Cure Alzheimer's Fund

Title: Brain substrate-specific transcriptional responses of human stem cell derived microglia

Authors: M. THERRIEN[1], ***T. ATKESON**[2], K. RAN[2], M. DOLAN[2], M. B. JOHNSON[3], B. A. STEVENS[4];
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Abstract: Microglia are the population of functionally and transcriptionally diverse macrophages of the central nervous system (CNS). As the main phagocytes of the brain, microglia are critical for clearance of debris generated by diseased cells through phagocytosis. Microglia engulf heterogeneously and abnormal engulfment is linked to neurodegenerative diseases including Alzheimer's disease. However, the transcriptional profile of phagocytic microglia remains relatively unexplored in humans. To investigate the transcriptional profile that drives microglia engulfment, we exposed human stem cell derived microglia to various brain-related substrates and identified transcriptional correlates of substrate-specific phagocytosis using single-cell RNA sequencing. We found gene expression pathways of human microglia exposed to apoptotic neurons, synapses, amyloid beta, and myelin correlate with microglia transcriptional states observed in disease. We validated the expression changes of a subset of these genes in independent microglia differentiations by qPCR. Our study will lay the groundwork for further engulfment assays in which candidate genes are up- or down-regulated and provide insight into phagocytosis as a potential therapeutic target.

Disclosures: M. Therrien: None. **T. Atkeson:** None. **K. Ran:** None. **M. Dolan:** None. **M.B. Johnson:** None. **B.A. Stevens:** None.

Poster

P192. Alzheimer's Disease: Genetics, Genomics, and Other Omics Approaches I

Location: Virtual Only

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Program #/Poster #: P192.09

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

Support: NIH Grant MH109260
NIH Grant T32 MH014654
Pennsylvania Department of Health Tobacco Settlement Act Grant RFA67-76
2017 NARSAD Young Investigator Grant #26634

Title: Differential gene expression and transcript usage at single-cell resolution in a mouse model of Alzheimer's disease

Authors: *A. E. WELLER, G. A. DOYLE, B. C. REINER, R. C. CRIST, W. H. BERRETTINI;
Psychiatry, Univ. of Pennsylvania Perelman Sch. of Med., Philadelphia, PA

Abstract: Alzheimer's disease (AD) is the most common form of dementia and is a source of significant morbidity and mortality for patients and their families. However, the specific cause of AD remains poorly understood. In humans, APOE4, an isoform of the *APOE* gene, is the strongest known genetic risk factor for late-onset AD. The *Apoe* knockout mouse has been used as a mouse model in the study of neurodegenerative diseases and is suitable for the study of AD. Single-cell (or single-nucleus) RNA sequencing (scRNAseq or snRNAseq) allows the analysis of the transcriptomes of single cells that can be used to identify cellular functions of various cell types, as well as differences in cellular transcriptomes between normal and disease states. SnRNAseq studies conducted in AD mouse models will aid in identifying the cells and genes with altered transcription that result in development of AD phenotypes. Using this rationale, we employed a publicly available dataset of snRNAseq data from 7 pooled hippocampi samples from 9-month-old C57BL/6J wild type (WT) mice and those from 7 9-month-old *Apoe* knockout (EKO) mice. We then used Seurat and Sierra programs to assess effects on differential gene expression and transcript usage, respectively, in hippocampal cell types mediated by constitutive *Apoe* gene knockout in mice. Seurat identified genes with differential expression between the WT and EKO samples by cell type (cluster). These cluster-specific genes were then used in Ingenuity Pathway Analysis (IPA). IPA identified 11 canonical pathway terms with FDR-adjusted p-values < 0.05, many of which have been implicated in AD. Additionally, Sierra identified several genes with differential transcript usage between WT and EKO samples, including *Sipa1l1*. The *Sipa1l1:* chr12:82235539-82235845:1 peak corresponded to a transcript variant (*Sipa1l1*-209) that was highly expressed in WT mice but had low levels of expression in the EKO mice. Furthermore, this *Sipa1l1* gene peak identified an alternative non-canonical polyadenylation signal and a putative cytoplasmic polyadenylation element (CPE). In humans, SIPA1L1 is a Rap GTPase-activating protein (RapGAP) involved in the regulation of dendritic spine morphogenesis in neurons. Our study data implicate differential transcript usage of *Sipa1l1* in CA1, CA2, and CA3 pyramidal cells; neurons known for having prominent dendrites and are

affected in neurodegenerative disorders such as AD, indicating that in humans SIPA1L1 may play a role in AD pathogenesis. Taken together, our results suggest that Apoe is involved in regulation of gene transcription and co-transcriptional RNA processing which may underlie AD.

Disclosures: A.E. Weller: None. **G.A. Doyle:** None. **B.C. Reiner:** None. **R.C. Crist:** None. **W.H. Berrettini:** None.

Poster

P229. Altered Energy Homeostasis in Alzheimer's Disease II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P229.01

Topic:

Support: AG057931

Title: Phenotypic characterization of an APOE mouse model of Alzheimer's Disease

Authors: *J.-P. WIEGAND, R. D. BRINTON;
Ctr. for Innovation in Brain Sci., Univ. of Arizona, Tucson, AZ

Abstract: Apolipoprotein E (APOE4) is the most prevalent genetic risk factor for Alzheimer’s Disease (AD), with a penetrance of ~50-60%. Transgenic APOE4-Alzheimer’s disease models are widely available, but many studies do not account for APOE4’s penetrance in their sample sizes. To address this gap, we assessed translational feasibility of the JAX APOE4 mouse model. We conducted population-level studies on monthly weights, survival rates, and clinical observations across early, midlife, and late development in APOE3/3, 3/4, and 4/4. Outcomes of these analyses indicated APOE4 mice consistently exhibited a divergence in weight at 15mo persisting throughout subsequent aging, showed significantly reduced survival rates, and more dermatitis, as compared to APOE3 controls. Thus, conclusions regarding metabolism, survival rates, and peripheral inflammation within the APOE4 mouse model advance translatability to human APOE4-AD.

Disclosures: J. Wiegand: None. **R.D. Brinton:** None.

Poster

P229. Altered Energy Homeostasis in Alzheimer's Disease II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P229.02

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

Support: NIH/NIA P01AG026572
NIH/NIA T32AG061897

Title: Breast Cancer Therapies Reduce Risk of Alzheimer's Disease and DementiaL Clinic to Bench Translation for Protective Mechanism

Authors: *G. L. BRANIGAN[1], G. TORRANDELL HARO[1], R. D. BRINTON[2];
[1]Pharmacol., [2]Ctr. for Innovation in Brain Sci., Univ. of Arizona, Tucson, AZ

Abstract: Background: Worldwide, breast cancer is the most common non-skin cancer in women. Approximately 1 in 8 women will be diagnosed with breast cancer during their lifetime. Currently, Alzheimer's disease (AD) affects 1 in 9 persons in the US over the age of 65, two thirds of whom are women. The high global prevalence and economic impact of AD presents a significant public health challenge while the identification of therapies to prevent AD remains a challenge. Previous studies have suggested that estrogen modulating therapies (EMT) used for the treatment of breast cancer are associated with a decreased risk of AD.
Methods: The objective of this study is to identify potential mechanisms by which EMTs alter biological pathways associated with AD prevention. E18 rat neurons cultures were treated with EMTs, vehicle or estradiol (E2) at clinically equivalent concentrations. The impact of EMT on neuron morphology was determined using MAP2 staining and confocal microscopy. Next, electrophysiologic recording were conducted using multi-electrode array (MEA). Finally, the impact of EMT on mitochondrial bioenergetics and dynamics were determined using Metabolic flux analysis.
Results: Data indicate that tamoxifen, exemestane and anastrozole treated neurons exhibit morphological features (e.g. increased cell area, dendrite length and branch patterns) comparable to E2 exposure when compared to vehicle ($p<0.001$) whereas raloxifene induced a less arborized structural phenotype ($p<0.01$). This trend was consistent across estrogen phenotypes in electrophysiologic (decreased interspike interval and increased burst firing) and mitochondrial bioenergetic parameters (increased mitochondrial respiration and ECAR) where tamoxifen and the aromatase inhibitors show similar responses to E2 when compared to vehicle ($p<0.001$) whereas raloxifene does not elicit an estrogen-like response.
Conclusion: Mechanistically, tamoxifen and raloxifene are known to act in a tissue-specific manner acting as receptor antagonists in breast tissue but show divergent actions in brain tissue. Results are suggestive that specific EMTs (tamoxifen) act as estrogen-like agonists in neurons. If tamoxifen and aromatase inhibitors are acting to increase estrogen-related actions in brain tissue, the argument for the protective association of estrogen with AD-related outcomes is strengthened.

Disclosures: G.L. Branigan: None. **G. Torrandell Haro:** None. **R.D. Brinton:** None.

Poster

P229. Altered Energy Homeostasis in Alzheimer's Disease II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P229.03

Topic:

Support: R37AG053589

Title: Dismantling of estrogenic control of the bioenergetic system in brain during female brain midlife aging: implications for preventing Alzheimer's disease

Authors: Y. WANG, F. YIN, ***R. D. BRINTON**;
Ctr. for Innovation in Brain Sci., Univ. of Arizona, Tucson, AZ

Abstract: Decline in brain glucose metabolism is a hallmark of late-onset Alzheimer's disease (LOAD). Chronological and endocrinological aging are associated with brain glucose hypometabolism and mitochondrial adaptations in female brain. Estrogen is a master regulator of the bioenergetic system in the female brain which requires integrating nuclear and mitochondrial genomic compartments. We sought to determine the process by which estrogenic control of the bioenergetic system in brain is dismantled during midlife endocrine aging. Using a rat model recapitulating fundamental features of the human menopausal transition, results of transcriptomic analysis revealed stage-specific shifts in bioenergetic systems of biology that were paralleled by bioenergetic dysregulation in midlife aging female brain. The first components of estrogenic control of bioenergetics to be dismantled were upstream transcription factors required for estrogenic control of mitochondrial gene expression. Consistent with early decline in NRF1 and TFAM, mitochondrial gene expression exhibited decline that paralleled the change in TFAM and its regulator NRF1. Mitochondrial-encoded oxidative phosphorylation (OxPhos) gene transcription rebounded, as did NRF1 and TFAM, which was followed by a significant decline which is in register with nuclear-encoded OxPhos genes. The second stage of components to be dismantled, that in part overlapped with the first wave, were genes required for estrogenic control of mitochondrial biogenesis and auxiliary fuel utilization. The third and final stage of components to be dismantled were transcription of genes required for OxPhos and encoded by both the nuclear and mitochondrial genomes. These findings indicate that the first dismantling point of estrogen regulation of the bioenergetic system is communication between the nuclear and mitochondrial genomes followed by dismantling of estrogen control of mitochondrial biogenesis which was followed by decline in transcription of both nuclear and mitochondrial genes required for oxidative phosphorylation required for ATP generation. Collectively, these results indicate that estrogenic control of the bioenergetic system in brain is systematically dismantled in stages. Further, these findings have implications for therapeutic intervention to prevent decline in glucose metabolism and mitochondrial function to prevent and reduce risk of Alzheimer's disease later in life.

Disclosures: Y. Wang: None. **F. Yin:** None. **R.D. Brinton:** None.

Poster

P229. Altered Energy Homeostasis in Alzheimer's Disease II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P229.04

Topic:

Support: NIA R01 AG057931
Women's Alzheimer's Movement

Title: Statins as Alzheimer's disease risk modifiers: Responder analysis for precision prevention

Authors: *G. TORRANDELL[1], G. L. BRANIGAN[3], R. D. BRINTON[2];
[2]Ctr. for Innovation in Brain Sci., [1]Univ. of Arizona, Tucson, AZ; [3]Pharmacol., Univ. of Arizona Hlth. Sci., Tucson, AZ

Abstract: Previously we reported reduced risk of Alzheimer's disease (AD) in persons treated with statins (Relative Risk [95% Confidence Interval]: 0.4643 [0.44-0.49]; *P* < .001) in a study population of 288,515 participants (Torrandell-Haro et al, 2020). Our current analysis aimed to validate these findings in a larger dataset and to identify responder vs non-responder phenotypes to statin therapy to advance precision medicine for prevention of AD and other age-related neurodegenerative diseases (NDD).A retrospective analysis was conducted using an insurance claims dataset including health and prescription records from 53 million people across the United States. Identified records included claims from persons 45 years or older and with no prior history of neurosurgery or NDD for analysis. An enrollment criterion which required that patients were enrolled in the dataset for at least 6 months before to first statin prescription and at least 3 years after was applied. A propensity score matching based on age, gender, cci and comorbidities was used to reduce bias in group assignment. Claims from 1,293,952 were surveyed for a diagnosis of AD and other age-related NDD 1 year after statin exposure. A series of sensitivity analyses were performed to compare the subset of responders to statin therapy to the non-responders group.Of the 1,293,952 participants included, 646,976 patients were treated with statin therapy, and 646,976 matched patients were not for the duration of analytic period. Statin use was associated with decreased risk of AD (RR [95% CI]: 0.54 [0.52-0.57]; *P* < .001), dementia (RR [95% CI]: 0.66 [0.64-0.68]; *P* < .001), non-AD dementia (RR [95% CI]: 0.72 [0.70-0.75]; *P* < .001), Multiple Sclerosis (RR [95% CI]: 0.20 [0.17-0.23]; *P* < .001), Parkinson's disease (RR [95% CI]: 0.39 [0.37-0.42]; *P* < .001), and ALS (RR [95% CI]: 0.46 [0.36-0.59]; *P* < .001). Relative risk analysis including sex differences indicated that men exhibited greater risk reduction benefit than women for AD, dementia, non-AD dementia and Parkinson's disease. Analysis of responders vs non-responders showed that the responder group was younger and overall healthier than the non-responders. Instead, women were predominant in the non-responder group.Results reported herein replicate previous findings of an association

between statin therapy and reduced risk of AD and other age-related NDD (Torrandell-Haro et al, 2020). Further, results of our current analyses characterize responders vs non-responders with women comprising the majority of non-responders. Outcomes of these analyses advance a precision medicine approach for preventing AD and multiple age-related neurodegenerative diseases.

Disclosures: G. Torrandell: None. **G.L. Branigan:** None. **R.D. Brinton:** None.

Poster

P229. Altered Energy Homeostasis in Alzheimer's Disease II

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Program #/Poster #: P229.05

Topic:

Support: NIH: R01AG057931
NIH: R01AG057931-01A1

Title: Infiltration of Peripheral Immune Cells in Human Brain During Mid-life

Authors: *H. VAN ROSSUM[1], A. MISHRA[1], N. DELATORRE[1], M. PADILLA-RODRIGUEZ[1], R. D. BRINTON[2];
[2]Ctr. for Innovation in Brain Sci., [1]Univ. of Arizona, Tucson, AZ

Abstract: Background: Inflammation is a well-documented feature of Alzheimer's disease (AD) in human brain. Two critical issues relevant to understanding the role of inflammation in AD that remain unresolved are: 1) the initiation phase of inflammation and 2) sex differences in the inflammatory cascade. Previous preclinical findings from our group indicated elevated immune transcripts including CD3, CD4, and MHCII. Herein, we extend and translate our preclinical discoveries to test the hypotheses that: 1) the inflammatory phenotype of LOAD is initiated in midlife of the human aging process and 2) the inflammatory cascade in brain is initiated by microglial activation in brain that promotes infiltration of peripheral immune cells into the brain. **Methods:** Both fixed and frozen human tissue samples were obtained from NIH NeuroBioBank (University of Maryland, University of Miami, and Mt. Sinai) that included the corpus callosum, hippocampus, and hypothalamus of 40 brain donors that were sex and age matched. For the purposes of this study, samples were sub-classified into 3 groups, healthy aging 40-49 years-old, healthy aging 50-59 years-old, and AD age 60-80 years-old. Neuro-inflammatory markers included microglia (IBA-1), activated microglia (HLA), and T-cells (CD3, CD4, CD8) were detected using 3,3'-diaminobenzidine immunohistochemistry. Transcriptomic analyses were conducted to confirm neuroinflammatory expression.
Results: Cell phenotyping analyses indicated presense of CD3+, CD4+ and CD8+ positive cells in human brain consistent with infiltration of peripheral T cells into brain. Magnitude of T-cell

indicators increased with age and was detectable in mid-life consistent with infiltration during midlife. In addition to CD3+, CD4+ and CD8+ T-cells activated and HLA+ microglia were present. **Conclusion:** These results provide evidence for early midlife infiltration of T-cells into brain which is coincident with activated microglial suggestive of immune signaling between the central and peripheral immune compartments. Outcomes of these and future analyses are relevant to effective immuno-therapeutics to target the earliest stages of AD to reduce risk and or delay AD.

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Poster

P229. Altered Energy Homeostasis in Alzheimer's Disease II

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Program #/Poster #: P229.06

Topic:

Support: R01AG057931
3R01AG057931-02S1

Title: Characterizing genetic susceptibility of white matter tracts and hippocampal volume within humanized APOE mice

Authors: *A. BHATTRAI, A. RAIKES, A. MISHRA, R. D. BRINTON;
Ctr. for Innovation in Brain Sci., Univ. of Arizona, Tucson, AZ

Abstract: Apolipoprotein (APOE) has been identified as one of the strongest risk factors associated with Late-Onset Alzheimer’s disease (LoAD). The *e*4 allele confers the highest susceptibility to developing cognitive dysfunction with females exbiting a two-fold higher incidence compared to males. LoAD MRI studies have shown a link between APOE-*e*4 gene load and reduced white matter integrity (WMI) in limbic and medial temporal regions as well as increased hippocampal volume loss (Bagepally, 2012). Female aging brain mouse models show catabolism of lipids and fatty acids, a potentially contributing factor to reduction in WMI (Klosinski, 2015). However, very few translational mouse models have utilized neuroimaging to identify the translatability of human LoAD etiology. We hypothesized that our hAPOE mice will exhibit reduced hippocampal volume and WMI, with more pronounced effects in e4 carriers and females.
APOE *e*3 and *e*4 genotype skull-extracted ex-vivo mouse brains (M/F; N=53; mean age = 17.95 month) were imaged (7T Bruker Biospec MRI scanner). Imaging sequences included isotropic structural and diffusion weighted imaging. Using the Dorr mouse brain atlas, bilateral hippocampal volumes and total brain volume (TBV) were extracted (Dorr, 2008). WMI was

quantified using fractional anisotropy (FA) whereas between-group comparisons were made using a groupwise connectometry approach (Yeh, 2013; Yeh, 2016).
Genotypic comparisons indicated lower mean FA values in APOE-*e*4 carriers (mean tractography FA =0.263+0.043) compared to the APOE-*e*3/3 carriers (mean tractography FA = 0.311+0.067) in a total of 8489 tracts (FDR corrected $p < 0.018$). Contrary to our hypothesis, females APOE-*e*4 carriers exhibited greater FA values (mean tractography FA = 0.289+0.036) compared to the males APOE-*e*4 carriers (mean tractography FA = 0.266+0.051) in a total of 5240 tracts (FDR corrected $p < 0.008$).
No between-genotype differences were observed for left or right hippocampal volumes; however, sex differences were observed for the right hippocampus with males having a significantly greater volume than females ($p = 0.007$). No such effect was observed for the left hippocampus.
MRI analyses indicate a significant reduction in FA values and in turn white matter integrity across the brain in APOE-*e*4 carriers, irrespective of sex. In line with human sex-differences literature, males exhibited greater total brain volume as well as greater right hippocampal volume compared to females, irrespective of genotype. These findings may provide evidence of deficits in lipid transport or lipid catabolism, resulting in decreased WMI in this hAPOE mouse model.

Disclosures: A. Bhattrai: None. **A. Raikes:** None. **A. Mishra:** None. **R.D. Brinton:** None.

Poster

P229. Altered Energy Homeostasis in Alzheimer's Disease II

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Program #/Poster #: P229.07

Topic:

Support: NIA Grant P01-AG026572
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R01 AG059093
Alzheimer’s Association SAGA-17-419459

Title: Mitochondrial gene transcriptional expression coupled with Alzheimers Disease risk factor in the aging brain

Authors: *Y. SHANG, F. YIN, R. D. BRINTON;
Ctr. for Innovation in Brain Sci., Univ. of Arizona, Tucson, AZ

Abstract: Mitochondrial dysfunction has been widely associated with various neurodegenerative diseases, yet the mechanisms regulating mitochondrial DNA (mtDNA) versus nuclear DNA (nDNA)-encoded genes in aging and Alzheimer’s disease (AD) brains are largely unknown. We used regression models to evaluate the association between mtDNA- and nDNA-encoded genes

at the transcript (ROSMAP, Mayo, MSBB datasets) and protein (ROSMAP) levels. The mtDNA- and nDNA-encoded oxidative phosphorylation (OXPHOS) genes were positively correlated at the protein level but were differentially regulated at the transcript level. In AD brains, while nDNA-encoded OXPHOS genes were downregulated, mtDNA encoded genes were mostly upregulated at transcript level. Furthermore, the transcriptional correlations between these two gene sets were reduced in AD brains. Using pathway analysis and cell-type deconvolution, we identified biological pathways and brain cell types that were correlated with mtDNA-encoded gene expression. In both normal and AD brains, mtDNA transcripts correlated positively with notch signaling, while negatively with synapse-, bioenergetics-, translation-, and ubiquitin-mediated protein degradation pathways. Neuronal cell fractions were negatively correlated with mtDNA transcripts whereas fractions of oligodendrocyte precursor cells, astrocytes, and pericytes exhibited positive correlations. Our analysis suggest an underappreciated correlation between the bigenome-controlled mitochondrial gene expression with the risk of AD. The crosstalk between mitochondrial genome and nuclear were reduced in AD, where nDNA-encoded OXPHOS genes were mostly down-regulated, suggesting potential therapeutic opportunities to rehabilitate mitochondrial function by targeting nDNA-encoded OXPHOS genes in AD brains.

Disclosures: Y. Shang: None. **F. Yin:** None. **R.D. Brinton:** None.

Poster

P229. Altered Energy Homeostasis in Alzheimer's Disease II

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Program #/Poster #: P229.08

Topic:

Support: R01AG057931
RF1AG059093
Center for Innovation in Brain Science

Title: Behavioral and metabolic characterization of a humanized APOE mouse model of Alzheimer's disease

Authors: *J. W. MCLEAN, A. BHATTRAI, J.-P. WIEGAND, A. RAIKES, R. D. BRINTON; Ctr. for Innovation in Brain Sci., Univ. of Arizona, Tucson, AZ

Abstract: Alzheimer’s Disease (AD) is the largest contributor to neurodegenerative dementia worldwide, with a correspondingly global impact upon communities, economies, and healthcare. AD is a complex systems biology challenge, with multiple pathways implicated ranging from immune response and lipid metabolism to cognitive function. This emphasizes AD’s multifactorial nature and the importance of knowing how AD risk factors interact with its

systems biology. Age is the greatest risk factor for AD and *APOE* polymorphism is the most important genetic risk factor for the predominant late-onset type of AD, where *APOE4* heterozygosity and homozygosity respectively confer three- and twelve-fold greater risk of developing AD compared to *APOE3* homozygosity. To address the translational validity of the Jackson Laboratory mouse model of AD with targeted replacement of mouse *Apoe* with either human *APOE3* or *APOE4*, behavioral and metabolic analyses were conducted in aged (18-25 months) mice of both sexes. Locomotive behavior, comprehensively assessed via open field analysis, revealed no sex or genotype differences; this observation was corroborated by no observed travel speed difference in the CatWalk assay. Additionally, cognitive performance in a novel object recognition (NOR) task was not measurably altered across genotypes or sexes. However, EchoMRI-based body composition analysis revealed significant ($p<0.05$) differences in adipose index (AI) between *APOE3* and *APOE4* aged mice. This was differentially regulated by sex, with male *APOE4* mice having the lowest AI, male and female *APOE3* mice having similar AI, and female *APOE4* having the highest AI. Another sex-dependent impact of the *APOE4* risk factor upon metabolism was observed in male *APOE4* mice exhibiting significantly ($p<0.01$) lower fasting blood glucose (FBG) than male *APOE3* mice, female *APOE3*, and female *APOE4* mice. However, *APOE* genotype did not influence female FBG. Lastly, ^{18}F-FDG-PET on a subset of these animals revealed less cerebral glucose uptake in *APOE4* females compared to males ($p=0.066$), highlighting a potential bioenergetic deficit where females have higher circulating blood glucose but decreased transport to the brain. No within-sex genotype effects were observed. Collectively, these results indicate that *APOE4* differentially impacts metabolism in male and female systems and follow-up metabolomics may potentially inform sex-based precision interventions. Additional ongoing correlational analysis between behavioral, metabolic, genetic, and sex components will help further apprise the strengths and weaknesses of this humanized *APOE* mouse model in AD-focused translational research.

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Poster

P229. Altered Energy Homeostasis in Alzheimer's Disease II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P229.09

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

Support: NIH Grant AG039452
NIH Grant AG023084
NIH Grant NS032267

Title: Generation and characterization of iPSC-derived cranial pericyte-like cells

Authors: *A. SAGARE[1], J. CHEN[2], K. KISLER[2], C. GRIFFIN[3], Z. DAI[2], B. ZLOKOVIC[2];
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Abstract: Brain vascular dysfunction contributes to the development of neurodegenerative disorders, including Alzheimer's disease (AD). We and others have shown that pericytes, which are vascular mural cells embedded in the wall of brain microvessels, play an essential role in the central nervous system (CNS) in regulating blood-brain barrier (BBB) integrity, controlling cerebral blood flow, clearing neurotoxins, providing neurotrophic support, and angiogenesis. Previous studies by our group have shown that pericyte degeneration and loss occurs in AD and that pericyte loss in the AD mouse model results in a significant increase in BBB leakage, increase in brain Aβ levels, tau pathology, and neuronal loss. The goal of the present investigation is to generate and characterize induced pluripotent stem cells (iPSC)-derived cranial pericyte-like cells (iPSC-PCs) by a two-step process via either mesoderm or neural crest cells intermediate for their potential use in a human patient-specific isogenic 3-D microfluidic system for CNS disease modeling studies and as a cell therapy treatment to repair and improve neurovascular functions in neurodegenerative disorders. Here using RNAseq and Western blotting analyses, we show that iPSC-PCs not only express pericyte specific proteins PDGFRβ, NG2, CD13, at similar levels as primary human adult forebrain pericytes, but also possess functional properties. The fully-characterized unlimited resource of iPSC-PCs that share molecular and phenotypic similarities with endogenous human adult brain microvascular pericytes hold potential in developing future non-neuronal pericyte-based cell therapy for AD and related neurodegenerative disorders.

Disclosures: A. Sagare: None. **J. Chen:** None. **K. Kisler:** None. **C. Griffin:** None. **Z. Dai:** None. **B. Zlokovic:** None.

Poster

P229. Altered Energy Homeostasis in Alzheimer's Disease II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P229.10

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

Support: Program for Neuropsychiatric Research, McLean Hospital

Title: Tackling Alzheimer's disease using a cellular platform

Authors: *K. C. SONNTAG, W. RYU, B. M. COHEN;
Psychiatry, McLean Hospital, Harvard Med. Sch., Belmont, MA

Abstract: Sporadic neurodegenerative disorders are complexly determined, with single causes rarely determining more than a modest proportion of risk. Nevertheless, key processes may be

identified that can be altered by targeting control points for entire pathways of pathology. Essential to understanding and targeting complexly determined disorders is the construction and application of suitable model systems. The cause of the progressive deterioration and death of neurons in sporadic or late-onset Alzheimer's disease (LOAD) is still poorly understood, but its pathology is multifactorial and closely related to various changes with age that are abnormally expressed in those at risk. To identify core pathogenic pathways and mechanisms and test therapeutic interventions, we developed a cellular platform consisting of skin fibroblasts derived from LOAD patients and AD-unaffected control individuals and therefrom generated induced pluripotent stem cells (iPSC) that are differentiated to brain-like cells, permitting to determine LOAD pathogenic processes in context of age, disease, genetic background, cell development, and cell type. To this end, the model has revealed that LOAD is associated with a multitude of bioenergetic and metabolic alterations, providing evidence for an innate inefficient cellular energy management in LOAD as a background to the development of neurodegenerative disease with age. Among the most striking bioenergetic anomalies in LOAD cells was a reduction of the redox reagent nicotinamide adenine dinucleotide (NAD) whose supplementation or increase has been suggested to reduce the risk of aging-associated conditions. To investigate NAD as a metabolic target, we used our platform to test nicotinamide riboside (NR), a dietary precursor for NAD *de novo* synthesis, and caffeine, which increases mitochondrial function and the expression of NMNAT2, an essential enzyme in NAD production. Supplementation with NR and caffeine partially restored NAD levels, however, did not correct the LOAD-associated bioenergetic phenotype. These data indicate that NAD alone may not be a sufficient control point to address dysfunctional energy management in LOAD, and they demonstrate the value of the platform in detecting abnormalities and testing interventions. Altogether, this cellular model allows for patient-oriented examination of numerous mechanisms and interactions in LOAD pathogenesis, which ultimately could be used as a basis for a personalized medicine approach to predict altered aging and risk for development of dementia, and to test or implement (customized) therapeutic or disease-preventive intervention strategies.

Disclosures: K.C. Sonntag: None. **W. Ryu:** None. **B.M. Cohen:** None.

Poster

P229. Altered Energy Homeostasis in Alzheimer's Disease II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P229.11

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

Title: ApoE4 confers Mitochondrial Substrate Oxidation defects that can be bioenergetically compensated by a Ketogenic substrate Beta-Hydroxy butyrate (BHB)

Authors: C. GARCIA[1], ***A. TOMILOV**[1], J. SANDOVAL[1], G. A. CORTOPASSI[2];
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Abstract: Background: Mitochondria are at the center of neural biogenergetics, and ApoE4 is the single most impactful risk factor for AD. We investigated the impact of ApoE on insulin sensitivity, on mitochondrial substrate utilization and bioenergetics. Persons with ApoE4 have reduced brain carbohydrate metabolism. To test for ApoE4 conferred neural mitochondrial metabolic differences, we constructed a novel stable-ApoE 2, 3 and 4 N2a cell model, and tested ApoE's effects on Insulin sensitivity, and mitochondrial glucose, lipid and ketone oxidation.
Method: Binding of ApoE isoforms E2, E3 and E4 to Insulin Receptor (IR) was measured by BLI and Co-IP, the impact of ApoE isoforms on mitochondrial glucose, lipid, and ketone oxidation was measured using Seahorse-XF.
Result: ApoE3 was found to sensitize to insulin about 2-fold more potently than ApoE4. ApoE isoforms directly bind Insulin Receptor; the binding constants were in the range of 200-300nM. Consistent with the previous insulin-sensitivity finding, ApoE3 caused a significant increase of the glycolytic rate and glucose oxidation relative to ApoE4. As there was no difference in oxidation of TCA cycle intermediates substrates in permeabilized cells, or isolated ApoE4 mitochondria we infer ApoE3's glucose advantage is the result of increased insulin sensitivity. ApoE4 contributed a significant palmitate oxidation defect relative to ApoE2 and ApoE3. We observed that the relative defect in ApoE4-dependent glucose and palmitate oxidation can be overcome by BHB. Thus, at the neural cell level, the metabolic defects contributed by ApoE4 appear to be rescued by a ketogenic molecule, that requires neither insulin nor apolipoprotein particle to reach neural mitochondria and provide alternative metabolic support.
Conclusion: ApoE4 confers 'double trouble' in mitochondrial glucose and lipid oxidation. ApoE4 confers a defect in mitochondrial lipid oxidation relative to all other isoforms. Simultaneously, ApoE4 lacks the benefit in glucose oxidation conferred by ApoE3, which appears to be driven by the reduced insulin sensitization potency of ApoE4. We also find that BHB can be an alternative source of neural bioenergy that enters mitochondria directly and thus is not affected by ApoE4 'double trouble'.
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Poster

P231. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P231.01

Topic:

Support: Scully Initiative
Taube Family Foundation
Jean Perkins Foundation
Horngren Family

Title: Dysregulation of activity-dependent alternative splicing events in tauopathy mice is normalized by in vivo modulation of p75 neurotrophin receptor signaling

Authors: *A. LATIF-HERNANDEZ[1], P. MORAN-LOSADA[1], T. YANG[1], K. C. TRAN[1], H. LIU[1], D. SIMMONS[1], S. M. MASSA[2], F. LONGO[1];
[1]Stanford Univ., Stanford, CA; [2]Dept. of Neurol., SFVAMC & UCSF, San Francisco, CA

Abstract: Tau pathology is associated with altered synaptic function, as measured by activity-dependent long-lasting changes of synaptic plasticity. Previously, we showed that synaptic dysfunction and changes in synapse-relevant gene expression profiles occur in the P301S (PS19) tauopathy mouse model and that treating with LM11A-31 (C31), a small molecule modulator of p75 neurotrophin receptor (NTR) signaling, normalized these abnormalities. Since neuronal activity produces changes in RNA splicing, we investigated whether mis-regulation of alternative splicing occurs in the context of plasticity in PS19 mice, and if so, whether it can be mitigated by C31. Wildtype (WT) and PS19 mice at 6 months of age were treated with vehicle or C31 (oral gavage) for 3 months. Acute hippocampal slices were prepared 24h after the last C31 dose and theta burst stimulation (TBS) was used to induce synaptic long-term potentiation [LTP]. RNA sequencing was performed on the non- [(-) TBS] and on the stimulated [(+) TBS] slices to generate activity-dependent profiles of alternative splicing events at the isoform level. Splicing analyses from (-) and (+) TBS slices in PS19 mice identified down-regulated isoforms enriched in *glutamatergic synapse, GABAergic synapse, calcium signaling, long-term potentiation,* and *axon guidance* KEGG pathways in (+) TBS slices relative to (-) TBS, suggesting specific activity-dependent changes at the isoform level. Further, comparison of (+) TBS PS19 and WT slices found that 1147 isoforms were significantly (FDR<0.05) altered in PS19 mice, with 264 down- and 883 up-regulated. C31 significantly decreased the total number of significantly altered isoforms in PS19 mice to 352, of which 158 were down- and 194 were up-regulated. Characterization of splicing events relevant to synapse dysfunction in tauopathy revealed that *glutamatergic synapse, calcium signaling, long-term potentiation,* and *axon guidance* were the most significant KEGG pathways associated with the down-regulated isoforms in PS19 mice, each of which were up-regulated in slices from PS19 mice treated with C31. Among these, we identified the following candidate components, via which C31 might restore synapse dysfunction: Gria1-202, Grin1-205, Grin2a-202, Cacna1h-203 and Lrrc40-206, which are implicated in signaling pathways crucial for synaptic transmission. Further exploration of these transcripts at the level of changes in protein isoforms is planned. In conclusion, modulation of $p75^{NTR}$ with C31 normalized tauopathy-related changes in synapse-relevant isoform levels suggesting that alternative splicing events regulated by C31 might contribute to neuronal plasticity in PS19 mice.

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Poster

P231. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P231.02

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

Support: National Institute of Neurological Disorders and Stroke (1R01NS106907 to A.K.S.)

Title: Neural Stem Cell-derived Extracellular Vesicle Therapy Slows Down the Progression of Disease in the 5XFAD model of Alzheimer’s disease

Authors: *M. LEELAVATHI NARAYANA[1], R. UPADHYA[1], S. ATTALURI[2], M. KODALI[1], L. MELISSARI[1], A. VOGEL[1], B. SHUAI[1], M. KHA[1], J. JAIMES[1], A. K. SHETTY[1]; [1]Inst. For Regen Med, Texas A&M Univ. Coll Med., College Station, TX; [2]Inst. For Regen Med. Texas A&M Univ. Coll Med., College Station, TX

Abstract: In the early stage of Alzheimer's disease, cognitive and mood impairments are typically associated with increased oxidative stress, neuroinflammation typified by activated microglia and astrocyte hypertrophy, and decreased hippocampal neurogenesis. These changes are also accompanied by a significant deposition of amyloid plaques in the brain. In the current study, using the 5XFAD mouse model, we examined the efficacy of intranasal (IN)treatment of extracellular vesicles (EVs) derived from the rat subventricular zone neural stem cells (SVZ-NSCs) for delaying the progression of AD. The EVs from the SVZ-NSC culture media were first purified by anion-exchange chromatography and then characterized using NanoSight and markers of EVs. Next, 3-months old 5XFAD mice received IN administration of EVs (once weekly for two weeks, 150 billion EVs/week) or the vehicle. Two months later, the animals were interrogated with a series of behavioral tests, following which the animals were euthanized, and the hippocampal tissues were examined for neuroinflammation, oxidative stress, neurogenesis, and amyloid plaque deposition. Compared to AD mice receiving the vehicle, EV-treated AD mice displayed improved cognitive function to discern minor changes in the environment in an object location test and better spatial recognition memory in an object-in-place test. EV-treated AD mice also displayed no anhedonia in a sucrose preference test. The functional benefits in EV-treated AD mice were associated with reductions in oxidative stress, neuroinflammation, and better neurogenesis in the hippocampus. Reduced oxidative stress was evident from lower

malondialdehyde and protein carbonyl levels and elevated catalase and superoxide dismutase levels. Diminished neuroinflammation could be gleaned from the reduced concentration of TNF-alpha and IL1-beta, diminished astrocyte hypertrophy, and reduced concentration of complement C3. In addition, microglia displaying activated NLRP3 inflammasomes and the extent of microglial cell clustering were reduced. Neurogenesis analysis using the birth-dating marker 5'-bromodeoxyuridine and the newly born neuron marker doublecortin revealed maintenance of a higher level of hippocampal neurogenesis in EV-treated AD mice compared to vehicle-treated AD mice. Notably, the extent of amyloid plaques was also reduced. Thus, intranasal administration of NSC-derived EVs in the early phase of AD has the promise to slow down the progression of AD.

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Poster

P231. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics I

Location: Virtual Only

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Program #/Poster #: P231.03

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

Support: AARFD-20-677771
NIH R01 AG060148

Title: Ablation of C5aR1 delays onset of hippocampal inflammatory gene expression and microglial activation in Arctic mice

Authors: *N. D. SCHARTZ, K. CARVALHO, G. BALDERRAMA-GUTIERREZ, H. YAHAN LIANG, S.-H. CHU, P. SELVAN, A. MORTAZAVI, A. J. TENNER;
Univ. of California, Irvine, Irvine, CA

Abstract: The Arctic APP transgene results in rapid accumulation of fibrillar amyloid plaques surrounded by dystrophic neurites which contribute to cognitive deficits and hippocampal neuronal loss by 10 months of age. Arctic mice also have increased gliosis and inflammatory gene expression in the brain at 5-10 months of age. As shown in our previous study, deletion of C5aR1 in the Arctic model (ArcticC5aR1KO) attenuates microglial activation, neuronal loss, and cognitive decline. To identify potential pathways that promote hippocampal injury, we assessed gene expression and microglia profiles of Arctic and ArcticC5aR1KO mice throughout disease progression. WT, C5aR1KO, Arctic, and ArcticC5aR1KO mice were aged to 2.7-10 months and hippocampi were isolated for bulk RNA-seq. MaSigPro was used to identify clusters of genes

that shared similar temporal expression patterns. In parallel, coronal sections from fixed brains of mice at 5, 7, and 10 months were stained for CD11c, CD11b, and AmyloGlo. Percent field area was analyzed with Imaris. We identified 15 clusters with maSigPro and compared the genes in those clusters with previously identified genes from Arctic microglia isolated from hippocampus and cortex and to bulk hippocampal sequencing from 5xFAD mice. Two of the clusters we identified contained many genes that were identified as upregulated in Arctic microglia and 5xFAD mice. Gene ontology of these two clusters identified inflammatory and cholesterol metabolism pathways. AD genes Cd33, Trem2, Tyrobp and Cst7 showed increased expression in the Arctic hippocampus with a peak at 7 months of age while in ArcticC5aR1KO the expression increased at a lower rate and did not peak until 10 months of age. Similarly, immunohistochemical quantification of CD11b- and CD11c-positive microglia showed an increase in these markers in Arctic mice compared to WT. While ArcticC5aR1KO mice also had higher levels compared to WT, at 7 months, CD11b and CD11c field area were lower compared to Arctic, suggesting a delay in microglia inflammatory activation. Early components of the classical complement pathway may aid in clearance of amyloid plaques and debris, while the downstream activation product C5a interacting with its receptor C5aR1 can cause inflammation and a feed-forward loop of neurodegeneration. By blocking only C5aR1, the beneficial functions of early complement remain intact, as does the lytic membrane attack complex which are useful for clearance of cellular debris and protection from infection. These findings provide support for C5aR1 as a potential drug target in the treatment of AD.

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Poster

P231. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics I

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Program #/Poster #: P231.04

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

Support: NIH AG 060148
NIH AG061746

Title: C5ar1 antagonist alters microglial cells in a mouse model of alzheimer's disease

Authors: *A. GOMEZ ARBOLEDAS, G. BALDERRAMA GUTIERREZ, P. SELVAN, S.-H. CHU, H. YAHAN LIANG, A. MORTAZAVI, A. TENNER;
Univ. of California Irvine, Irvine, CA

Abstract: Alzheimer's disease (AD) is a devastating neurodegenerative disorder that impairs memory and causes cognitive decline and memory loss. The etiology underlying late onset AD is very complex and multi-factorial, where inflammation and the complement system are known to be essential contributors. Activation of the complement system by fAβ (and by neurofibrillary tangles) can generate C5a, that binds to its receptor C5aR1 on microglial cells and contributes to neuroinflammation. Previous results from our lab have shown a beneficial effect using either a genetic ablation of C5aR1 (C5aR1KO) or a pharmacological inhibition of it (C5aR1 antagonist, PMX205) in AD mouse models. Here, we further examined the effect of PMX205 on microglial gene expression and gliosis in a mouse model of Alzheimer's disease. Tg2576 mice (and WT littermates) have been treated with PMX205 in the drinking water for 12 weeks. Mice were perfused and brains were harvested and further processed for single cell RNA-sequencing and immunohistochemical analysis. As expected, an intense microglial response, demonstrated by enhanced Iba1 staining, increased with age and amyloid pathology. However, Tg2576 treated with PMX205 showed a reduced expression of multiple microglial markers, such as Iba1, CD11b or CD11c (37%, 65% and 82% reduction in the hippocampus). Moreover, single cell RNAseq analysis revealed significant changes in the proportions of microglial clusters when AD animals were treated with PMX205. Homeostatic microglial clusters were downregulated in AD mice, while Disease Associated Microglial clusters (characterized by the expression of ApoE, Tyrobp, Cst7, Csf1 and Axl, among others) were enhanced after the treatment with PMX205. Of the 9 microglial clusters identified, 3 were reduced with PMX205 while 3 others were enhanced by PMX205 treatment. Our results are consistent with the pharmacological inhibition of C5aR1 in a mouse models of Alzheimer's disease being linked to polarizing the disease associated microglia towards disease mitigating vs disease enhancing and is reflected in reduced neuroinflammatory pathology. Thus, modulation of C5a-C5aR1 could be a valuable therapeutic strategy for Alzheimer's disease patients.

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Poster

P231. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics I

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Program #/Poster #: P231.05

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

Support: NIH GRANT 2R44AG053150-05

Title: Acute dosing with a small molecule inhibitor of tau self-association in tau P301L JNPL3 mice to identify pharmacodynamics readouts.

Authors: *D. PATEL[1], C. SUSSMAN[1], M. GRECO[1], P. LOPEZ[1], E. DAVIDOWITZ[1,2], J. MOE[1,2];
[1]Oligomerix, Inc., 1300 Morris Park Ave., Room 1211, Bronx, NY; [2]Oligomerix, Inc., 2 Westchester Park Drive, Suite 208, White Plains, NY

Abstract: There is a critical unmet need for a disease modifying therapeutic for Alzheimer's disease (AD). Key requirements for treating early-stage AD include safe, efficacious, and cost-effective therapeutic interventions. Presently, we have developed a novel series of small molecule inhibitors of tau self-association (TSA). Our target approach is agnostic to the multiple strains of tau aggregates that form in disease, and to mutations in tau associated with tauopathies and is highly differentiated from other programs that target fibril formation or tangle disaggregation at the end of the aggregation cascade. Earlier, we reported the in vivo preventive efficacy of our small molecule lead in two tau-based transgenic mice models (htau mice; Davidowitz et al., 2020 and human tau P301L JNPL3 mice). The tau P301L mutation found in the inherited forms of progressive supranuclear palsy (PSP) and frontotemporal dementia (FTD) causes tau aggregation and neurodegeneration. In female homozygous JNPL3 mice, expression of the human P301L tau 4R0N construct in the hindbrain and spinal cord causes hind limb motor impairment to develop with age. The objective of this study is to identify acute pharmacodynamic (PD) measures of efficacy that will ultimately be used for modelling PK/PD relationship of our inhibitors. Acute dose range-finding studies were performed using multiple routes of administrations. C57BL/6 (WT) mice (n = 30; 15 males, 15 females) were administered vehicle (n = 3 mice/gender) or a single dose of TSA inhibitor ranging from 2 to 200 mg/kg (n = 3 mice/gender/dose). Brain and whole-blood specimens were collected at one-hour post-dose. Samples of serum and brain homogenates are being analyzed to evaluate compound concentrations by LC-MS/MS. PK studies in male and female WT mice will be performed with and without fasting to determine the food effects on bioavailability. The results of this study will be used to select the dose and route of administration for PK studies in homozygous female JNPL3 mice aged to 9 months. To determine the selective dose for the PD evaluation, PK studies will be performed using three doses. Some of the PD measures that will be evaluated are tau self-association, tau hyperphosphorylation, and motor behavior. In summary, understanding PK/PD relationships from this study will be crucial for determining appropriate dosing levels of TSA inhibitors in future clinical studies for AD and related tauopathies.

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Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Oligomerix, Inc. **J. Moe:** A. Employment/Salary (full or part-time):; Oligomerix, Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Oligomerix, Inc.

Poster

P231. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics I

Location: Virtual Only

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Program #/Poster #: P231.06

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

Support: ADDF-T-PEP-18-579974C
NIH/NIA-RO1 AG059799

Title: Nsmase2 inhibition reduces phosphorylated tau in mouse model of alzheimer's disease

Authors: *K. COLEMAN[1], B. S. SLUSHER[2], B. J. BELL[3], C. TALLON[4], T. JOE[4];
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Abstract: Alzheimer’s Disease (AD), the most common form of dementia, currently affects 50 million people and is set to increase substantially with the aging population, yet there remain no effective treatments. Therefore, it is imperative to explore new targets and mechanisms for slowing or halting the progressive propagation of toxic hyperphosphorylated tau that likely contributes to the neurodegenerative process. One such approach is by targeting extracellular vesicles (EVs). These small cargo-containing vesicles have been demonstrated to transport phosphorylated tau species in AD brains, enabling its propagation from affected to naïve neurons. Previously, our lab and others have shown that genetic knockdown or pharmacological inhibition of the enzyme neutral sphingomyelinase 2 (nSMase2) reduces the biogenesis and release of EVs. nSMase2 catalyzes the hydrolysis of sphingomyelin into ceramide, a necessary component of EV synthesis. nSMase2-inhibiting tool compounds, such as GW4869, have shown promise in ameliorating the buildup of toxic tau species in mouse models of AD, but they have poor potency, solubility, and limited CNS penetrance. Given the significant clinical potential of this target, our lab conducted a high throughput screening campaign of over 400,000 compounds against human nSMase2 followed by extensive medicinal chemistry efforts which identified 1-(3-(3,4-dimethoxyphenyl)-2,6-dimethylimidazo[1,2-b] pyridazin-8-yl) pyrrolidin-3-yl) carbamate (PDDC), a potent nSMase inhibitor (IC_{50}=300 nM) wth excellent oral bioavailability (F%=87) and brain penetration (AUC_{brain}/AUC_{plasma}=0.6). PDDC has been shown to inhibit the release of EVs in a dose-dependent manner in both *in vitro* and *in vivo*. To examine the

therapeutic potential of PDDC for AD, we dosed 4-month old PS19 mice for 5 months with PDDC-containing chow at a dose equivalent to 100 mg/kg/day which consistently delivered PDDC concentrations to the brain > its nSMase2 IC_{50}. After 5 months of therapy, we sacrificed the mice and extracted protein from the hippocampus and cortex. Total, pThr181, pSer202/Thr205, and pThr217 phosphorylated tau levels were quantified via western blot and compared to age and sex-matched control mice treated with vehicle chow. We observed a significant reduction in total and pThr181 tau in mice treated with PDDC compared to controls. pSer202/Thr205 and pThr217 were also lower in treated animals, however, these did not reach significance. These results demonstrate PDDC is capable of reducing pathological tau burden further supporting the clinical relevance of SMase2 inhibition for the treatment of AD.

Disclosures: K. Coleman: None. **B.S. Slusher:** None. **B.J. Bell:** None. **C. Tallon:** None. **T. Joe:** None.

Poster

P231. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P231.07

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

Support: BrightFocus Foundation

Title: Altered sodium channel inactivation status in a Drosophila model of Alzheimer's disease

Authors: V. SANCHEZ FRANCO, A. N. HUTSON, S. D. DANIELS, *M. TABUCHI; Dept. of Neurosciences, Case Western Reserve Univ. Sch. of Med., Cleveland, OH

Abstract: While neuronal hyperexcitability has been implicated in the etiology of Alzheimer's disease (AD), its mechanistic causality is poorly understood. Not only has early neuronal hyperexcitability of cortical inhibitory interneurons been identified in mammalian models of AD, but the synergistic interaction between sleep loss and AD pathogenesis to further enhance neuronal hyperexcitability has also been demonstrated. Previous research using a Drosophila model of AD, which allows for genetic manipulations in specific cell types, has shown altered intrinsic membrane properties due to decreased conductance of voltage-gated potassium channels. Here we found that in addition to voltage-gated potassium currents, voltage-gated sodium currents have also been altered in a Drosophila model of AD. Specifically, we found that sodium channel inactivation status was significantly prolonged in Drosophila neurons expressing human β-amyloid (Aβ) and Tau. Further, we found that this prolonged sodium channel inactivation was synergistically enhanced by chronic sleep deprivation, suggesting an exacerbation loop between sleep and AD pathogenesis in shaping neuronal hyperexcitability.

Strikingly, we also observed that providing Drosophila with both Levetiracetam (LEV) and brivaracetam (BRV) within food negated the prolongation of the sodium channel inactivation status. Together, our results indicate that changes in sodium channel inactivation could be a novel predictor to suppress neuronal hyperexcitability induced by sleep and AD, which has the potential to reduce attrition in the late phases of neuroprotective medication development.

Disclosures: V. Sanchez Franco: None. **A.N. Hutson:** None. **S.D. Daniels:** None. **M. Tabuchi:** None.

Poster

P231. Therapeutic Strategies: Preclinical Animal Models and Small Molecule Therapeutics I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P231.08

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

Support: NSF Grant HRD-1905148

Title: The effects of presenilin knockdown on longevity, learning, and memory in a Drosophila melanogaster model of Alzheimer's disease

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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disease that results in decline in cognition and behavior. Considering the impact AD has on individuals, there is a need to study the disease from multiple perspectives. This study is designed to characterize the effects of presenilin dysfunction, a gene that is the most common cause of familial AD, in our *Drosophila melanogaster* model of AD. Presenilin-1 loss of function is thought to underlie the pathogenic beta-amyloid production. Drosophila melanogaster has a homolog of presenilin, dPsn, and our study examines the effect of dPsn loss of function via knockdown on the fruit fly's longevity and spatial cognition. We first generated three lines that knocked down the expression of dPsn using dsRNA of dPsn. These lines included knockdown either pan-neuronally, in the mushroom bodies, or ellipsoid body, as the latter two have been shown to be important in learning, memory and spatial cognition. To assess the effect on lifespan, flies from each line were collected and the number of deaths recorded each day. The knockdown of dPsn in the fruit fly had a demonstrable effect on longevity and resulted in a shortened lifespan. A visual place learning assay was used to examine the cognitive abilities and behavior in the fruit fly. Flies were trained to locate a hidden cool spot on a hot surface using visual cues and were then tested for memory of that location. Flies were assessed on learning over ten trials and memory for up to one hour after training. The knocked down expression of dPsn in different brain areas had a

noticeable impact on the fruit flies' learning and memory. These findings suggest knockdown of dPsn in the fruit fly resulted in an observable effect on longevity, health, and behavioral deficits. Specifically, the ability to quantify changes in spatial cognition helps advance the fruit fly model of AD to better reflect the cognitive decline observed in humans.

Disclosures: K.M. Estes: None. **M.D. Charles:** None. **E. Woo:** None. **D.D. Lent:** None.

Poster

P238. Therapeutic Strategies: Preclinical Animal Models and Peptide-Based Therapeutics

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P238.01

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

Title: Rescue of lysosomal storage disorder phenotypes caused by Grn loss of function with a brain penetrant progranulin biologic

Authors: *M. J. SIMON, T. LOGAN, A. RANA, G. M. CHERF, S. S. DAVIS, R. L. Y. LOW, C.-L. CHIU, M. FANG, F. HUANG, A. BHALLA, M. PIZZO, J. HSIAO-NAKAMOTO, Y. RAJENDRA, J. WANG, Y. ROBLES-COLMENARES, D. KIM, H. NGUYEN, T. K. EARR, B. TSOGTBAATAR, R. CHAU, R. RAVI, R. MEISNER, D. DIAZ, K. R. HENNE, K. A. SCEARCE-LEVIE, R. WATTS, J. H. SUH, J. W. LEWCOCK, S. L. DEVOS, G. DI PAOLO; Denali Therapeut., South San Francisco, CA

Abstract: Mutations in the Grn gene are a risk factor for frontotemporal dementia (FTD), with progranulin (PGRN) haploinsufficiency observed in 10-15% of all FTD cases (GRN-FTD). Complete PGRN loss-of-function is linked to the lysosomal storage disorder Neuronal Ceroid Lipofuscinosis, which is defined pathologically by CNS lipofuscin deposition. Various lysosomal functions of PGRN have also been described preclinically including pH maintenance and lipase activity regulation. Based on these genetic data and the similarities between PGRN loss-of-function and lysosomal storage disorders, we hypothesize that CNS-penetrant PGRN replacement approaches represent a viable therapeutic mechanism for treating PGRN deficiency phenotypes. Using a mouse model of PGRN deficiency (Grn KO mice), here we further profile the role of PGRN in regulating lysosomal lipid composition and lipase activity. We also evaluate the viability of a brain penetrant, recombinant progranulin fusion protein as a potential therapeutic approach. To achieve this, we utilized our investigational therapeutic, PTV:PGRN, which is composed of recombinant human PGRN, fused to an Fc fragment engineered to contain a transferrin-receptor (TfR) binding site, that facilitates blood brain barrier transcytosis.Using a broad-panel lipidomic/metabolomic approach, we found that Grn KO mice exhibit age-independent deficiency of the lysosomal phospholipid bis(monoacylglycero)phosphate (BMP), and age-dependent accumulation of the sphingolipid glucosylsphingosine (GlcSph). Next, using Grn KO mice expressing humanized transferrin receptor (Grn KO x $TfR^{mu/hu}$ KI), we

demonstrate that PTV:PGRN exhibits superior brain uptake relative to a non-TfR binding PGRN molecule and rapid correction of brain BMP deficiency. We also demonstrate across a dose range, a single dose of PTV:PGRN can correct brain BMP and GlcSph phenotypes for up to 6 weeks. Finally, we show with repeat dosing, PTV:PGRN also rescues neuroinflammatory and neurodegenerative phenotypes observed in Grn KO mice (elevated gliosis marker expression, lipofuscin deposition and CSF Nf-L accumulation). Our data further support the link between PGRN loss of function and lysosomal lipid dysregulation and illustrate that certain lipid phenotypes can be targeted with PTV:PGRN. Moreover, the data suggest that lysosomal phenotypes may underlie glial activation and neuronal degeneration observed in Grn KO mice, which are also rescued with PTV:PGRN treatment. Taken together our data provide preclinical proof of concept for PTV:PGRN, supporting its use as a potential therapeutic in the treatment of PGRN loss-of function and GRN-FTD.

Disclosures: M.J. Simon: A. Employment/Salary (full or part-time):; Denali Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Denali Therapeutics. **T. Logan:** A. Employment/Salary (full or part-time):; Denali Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Denali Therapeutics. **A. Rana:** A. Employment/Salary (full or part-time):; Denali Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Denali Therapeutics. **G.M. Cherf:** A. Employment/Salary (full or part-time):; Denali Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Denali Therapeutics. **S.S. Davis:** A. Employment/Salary (full or part-time):; Denali Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Denali Therapeutics. **R.L.Y. Low:** A. Employment/Salary (full or part-time):; Denali Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Denali Therapeutics. **C. Chiu:** A. Employment/Salary (full or part-time):; Denali Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Denali Therapeutics. **M. Fang:** A. Employment/Salary (full or part-time):; Denali Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Denali Therapeutics. **F. Huang:** A. Employment/Salary (full or part-time):; Denali Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Denali Therapeutics. **A. Bhalla:** A. Employment/Salary (full or part-time):; Denali Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Denali Therapeutics. **M. Pizzo:** A. Employment/Salary (full or part-time):; Denali Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Denali Therapeutics. **J. Hsiao-Nakamoto:** A. Employment/Salary (full or part-time):; Denali Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Denali Therapeutics. **Y. Rajendra:** A. Employment/Salary

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Poster

P238. Therapeutic Strategies: Preclinical Animal Models and Peptide-Based Therapeutics

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P238.02

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

Support: FAPERJ 202.432/2014 (Ferreira)
INNT 465346/2014-6(Ferreira)
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International Society for Neurochemistry (Selles)
IUBMB Wood-Whelan Fellowship (Selles)
PEW Latin American Fellowship (Selles)

Title: Chronic intranasal oxytocin treatment restores social memory loss in the APPswe/PS1dE9mouse model of Alzheimer's disease

Authors: *M. SELLES[1], J. T. S. FORTUNA[2], Y. P. R. DE FARIA[2], B. M. LONGO[3], R. C. FROEMKE[4], M. V. CHAO[5], S. T. FERREIRA[6];
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Abstract: Alzheimer's disease, the main cause of dementia in the elderly, is accompanied by social memory deficits. Oxytocin is a pro-social neuropeptide that has recently been shown to be neuroprotective. Here, we characterized oxytocin production in the APPswe/PS1ΔE9 mouse model of Alzheimer's disease, and evaluated the potential of oxytocin to correct social memory impairments in those mice. Therefore, we used qPCR to quantify oxytocin mRNA levels in the hypothalamus of 12 month old male mice (n=7-9 mice per group) and observed a decrease trend in the levels of oxytocin in APPswe/PS1ΔE9 compared to wild type mice (p=0.08). Aiming to increase cerebral oxytocin levels, we developed and validated a protocol to deliver oxytocin via intranasal in mice. This protocol was efficient in increasing oxytocin peptide levels in the hippocampus of mice (p=0.05), and to reduce fear response in the fear conditioning paradigm (p=0.03) – a known effect induced by central oxytocin (n=10-15 mice per group). Next, we implemented this protocol to evaluate the consequences of oxytocin on social memory in APPswe/PS1ΔE9 using the 5-trial social task. APPswe/PS1ΔE9 treated with oxytocin were protected against social memory loss, which was present in APPswe/PS1ΔE9 treated with

vehicle (n=14-15 per group, $p<0.01$). Together, our results suggest that oxytocin could be a potential therapeutic approach to correct social memory deficits in Alzheimer's disease.

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Poster

P238. Therapeutic Strategies: Preclinical Animal Models and Peptide-Based Therapeutics

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P238.03

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

Title: Bdnf reduces aβ in 3d cultures of alzheimer's disease

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Genet. and Aging Res. Unit, McCance Ctr. for Brain Health, MassGeneral Inst. for Neurodegenerative Dis. (MIND), Dept. of Neurology, Massachusetts Gen. Hosp. and Harvard Med. School, Charlestown, MA, 02129, USA, Boston, MA

Abstract: Alzheimer's disease (AD) is an irreversible, progressive neurological disorder that is characterized by memory loss and cognitive disturbance. Two pathological hallmarks of AD are: 1) amyloid plaque, primarily composed of amyloid-β (Aβ) which is derived from the amyloid precursor protein (APP) via serial cleavage of β- and γ-secretase; and 2) neurofibrillary tangle which is composed of filamentous accumulations of aggregated hyperphosphorylated tau (pTau). Brain-derived neurotrophic factor (BDNF) is a member of the nerve growth factor family and has been the focus of intense interest in AD research. While it is widely accepted that BDNF has an important role in modulating cognitive function as well as synaptic and neuronal dysfunction, the direct roles of BDNF on Aβ and tau pathologies are unknown or inconclusive. We previously developed a three-dimensional (3D) "human" neural cell culture model of AD which displays robust Aβ deposits and tauopathy (referred as 3D cultures; *Choi et al.,* Nature 2014). Here, we found that BDNF treatment significantly reduced the levels of Aβ40 and Aβ42 as well as Aβ42/Aβ40 ratio in the conditioned media of 3D cultures that harbor APP Swedish/London mutations. BDNF also reduced the levels of APP C-terminal fragment α and β (APP-CTFα and APP-CTFβ, respectively). Further mechanism study is warranted.

Disclosures: G. Bakiasi: None. **M. Panier:** None. **B. Gong:** None. **R.E. Tanzi:** None. **S. Choi:** None.

Poster

P238. Therapeutic Strategies: Preclinical Animal Models and Peptide-Based Therapeutics

Location: Virtual Only

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Program #/Poster #: P238.04

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

Support: Reed - U54GM104942
Reed - R15AG045812
Reed - NIRG-12-242187

Title: Modifying glutamatergic neurotransmission rectifies synaptic plasticity and memory deficits in a 3xTg Alzheimer's disease model

Authors: *J. PFITZER[1], P. D. PINKY[2], I. A. QURESHI[4], R. M. BERMAN[4], V. D. SUPPIRAMANIAM[3], M. REED[5];
[2]Drug Discovery and Develop., [3]Harrison Sc Pharm., [1]Auburn Univ., Auburn, AL; [4]Biohaven Pharmaceuticals, New Haven, CT; [5]Aubrun Univ., Auburn, AL

Abstract: Alzheimer's disease (AD) poses a public health crisis with an urgent need for effective treatments and disease-modifying therapies. Glutamate dysfunction is a core feature of AD, and glutamatergic neurotransmission is a validated target for treating AD. In this preclinical study, we examined the effects of the novel glutamate modulator, troriluzole, on synaptic function, memory deficits, and glutamate release using the triple transgenic (3xTg) AD model. 3xTg mice received the troriluzole (25 mg/kg/day) from 5-8 months of age. At eight months of age, drug-treated 3xTg mice were compared to vehicle-treated 3xTg and transgene negative (TgNeg) controls for alterations in behavioral memory, synaptic plasticity, and glutamate release. Treatment attenuated the hippocampal-dependent reference memory deficits observed in 3xTg mice in Morris water maze probe trials. Treatment also the attenuated basal synaptic transmission deficits seen in 3xTg mice. Paired-pulse facilitation (PPF), a measure of the probability of glutamate release, was significantly reduced in vehicle-treated 3xTg slices, indicating an increase in presynaptic glutamate release probability; drug treatment restored PPF in 3xTg mice to that of controls. We also observe an increase in KCl-evoked hippocampal glutamate release in the of 3xTgs that was brought toward that of controls with drug treatment. The amount of readily releasable pool (RRP) of synaptic vesicles was also significantly reduced in 3xTg mice, indicating increased vesicle docking, and treatment significantly increased RRP in 3xTg mice. Long-term potentiation, a cellular correlate of learning and memory, was reduced by 25% in vehicle-treated 3xTg mice compared to controls. Notably, drug treatment completely restored the synaptic plasticity deficits observed in 3xTg mice. Together, these results suggest that troriluzole may restore synaptic deficits in 3xTg mice via modulation of glutamatergic neurotransmission and highlight its potential as a novel therapy for AD.

Disclosures: J. Pfitzer: None. **P.D. Pinky:** None. **I.A. Qureshi:** None. **R.M. Berman:** None. **V.D. Suppiramaniam:** None. **M. Reed:** None.

Poster

P238. Therapeutic Strategies: Preclinical Animal Models and Peptide-Based Therapeutics

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P238.05

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

Title: A Novel sulfono-γ-AApeptide, 125-6b, as Therapeutic for Alzheimer's Disease

Authors: *N. SHEN[1], L. WEI[2], H. YANG[1], Y. WANG[1], J. KIM[1], T. ZIENELDIEN[1], J. CAI[2], C. CAO[1];
[1]Taneja Col. of Pharm., [2]Chem., Univ. of South Florida, Tampa, FL

Abstract: Mounting evidence suggests that the accumulation and aggregation of Aβ peptides are major and early etiological factors for Alzheimer's disease (AD), and the Aβ oligomers and fibrils are the major cause of AD due to their toxicity to neurons. Thus, the anti-Aβ aggregation approach has more potential to become a therapeutic treatment of AD, compared to the approach that targets the monomeric Aβ, which is believed to have an important physiological function. 125-6b is a sulfono-γ-AApeptides, which mimics the α-helical domain of proteins and could prevent degradation by proteases. This peptide mimic is one of the series of the helical sulfono-γ-AApeptides that stabilizes monomeric helical Aβ and potently inhibit Aβ aggregation and further aim to prevent the development or reverse AD. Our results demonstrated that 125-6b could inhibit Aβ aggregation, enhance neuronal survival and mitochondria function, and dramatically improved the memory of mice in the behavior test. The anti-aggregation result has been captured by the western blot assay. N2a/APP cell line and the primary neuron of newborn mice were treated with 125-6b and used to observe cell growth and mitochondria function. Aged APP/PS1 mouse model was used for the drug efficient test by intranasal delivery for 2.5 months. All the mice were evaluated by Radial Arm Water Maze (RAWM) and plasma Aβ levels before and after the treatment. 125-6b could significantly inhibit the Aβ aggregation, and promote the cell growth of both N2a/APP and primary neuron cell confirmed by MTT and Cell Titer-Glo Assay. Compound 125-6b could enter cell mitochondria, and increase the mitochondrial function. *In vivo,* post-treatment RAWM results demonstrated that 126-6b can statistically significantly improved the vulnerability of transgenic mice. The plasma Aβ40 and 42 levels had no differences among transgenic groups, however, the Aβ42/40 ratio increased with treatment. The mechanism of the drug is still undergoing further research, and this peptidomimetic has outstanding potential to be a therapeutic drug for AD.

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Poster

P238. Therapeutic Strategies: Preclinical Animal Models and Peptide-Based Therapeutics

Location: Virtual Only

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Program #/Poster #: P238.06

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

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John and Patricia Beckler Fellowship in Alzheimer's and Cognitive Diseases Fellowship

Title: Molecular effects of intranasal insulin in the rodent brain: Examination of insulin receptor signaling and the glutamatergic system

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Abstract: As brain insulin resistance has been identified as a pathological feature of age-related cognitive decline (ARCD), intranasal insulin (INI) is being explored as a potential treatment for patients with ARCD. Previous studies have demonstrated that INI enhances memory, but the underlying mechanisms remain unclear. To investigate the mechanistic basis for the pro-cognitive effects of INI, insulin receptor (IR) signaling and the expression of glutamate receptors and transporters, due to the role of the glutamatergic system in hippocampal synaptic transmission, was examined. Adult male Sprague-Dawley rats received bilateral hippocampal injections of a control lentiviral vector (LV-Con) or one containing a selective insulin receptor antisense sequence (LV-IRAS) to induce hippocampal-specific insulin resistance. Seven months later, *in vivo* microdialysis was used to assess brain neurochemistry and on a separate day, these animals were administered INI 30 minutes before euthanasia. In another cohort of Fischer 344 x Brown Norway F1 hybrid male rats (no virus) we investigated the effects of acute and chronic (10 days) INI dosing paradigms in young (3 months old) and aged (26 months old) rats, with a final INI administration 30 minutes before euthanasia. In both experiments, the hippocampus was processed for immunoblot analysis to assess changes in central IR signaling and phosphorylation/expression of glutamate receptor subunits and transporters. We previously observed that LV-IRAS injection selectively downregulated hippocampal IR expression and signaling without affecting peripheral insulin sensitivity. In this study, LV-IRAS animals showed reduced hippocampal basal glutamate levels and decreased phosphorylation/expression of glutamate receptor subunits. Additionally, vesicular glutamate transporter 2 (vGluT2)

expression, but not vGluT1, was significantly decreased in the hippocampus of LV-IRAS animals after INI administration. Immunofluorescence analysis was also used to determine that LV-IRAS animals exhibited decreases in the major presynaptic vesicle protein in the hippocampus. In the other cohort, age- and dosing paradigm-dependent effects were observed vis-à-vis IR signaling and glutamate receptor phosphorylation/expression. These data indicate that INI may improve cognition through the enhancement of IR signaling in the brain and/or through exerting synaptic effects on glutamate neurotransmission. More studies are needed to fully understand the mechanistic changes following INI and to determine the most effective treatment strategies, as INI could eventually be used in the broader clinical setting to treat ARCD.

Disclosures: J.M. Erichsen: None. **J.L. Woodruff:** None. **J.N.J. Crawford:** None. **C.A. Grillo:** None. **L.P. Reagan:** None. **J.R. Fadel:** None.

Poster

P238. Therapeutic Strategies: Preclinical Animal Models and Peptide-Based Therapeutics

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P238.07

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

Support: This work was predominantly funded by the Alan and Janice Woll Foundation.

Title: Identifying the multifactorial therapeutic mechanisms of irisin against emerging neuropathology in htau and 3xtg mice

Authors: *K. A. BRETLAND[1], K. M. BRETLAND[2], L. LIN[1], S. TERRILL[1], G. LEONE[1], C. M. DENGLER-CRISH[1];
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Abstract: Alzheimer’s disease (AD) is an age-related neurodegenerative disorder that continues to exact a disabling and lethal toll on patients. Late-onset AD, the most common form of dementia, has a multifactorial etiology that has challenged the development of disease-modifying drugs due to the need for these agents to address multiple mechanisms. However, the recently-discovered exercise-evoked hormone irisin could provide a unique therapeutic opportunity to address all of the key pathologies seen in AD: amyloid-beta plaques, neurofibrillary tangles, and neuroinflammation, as well the major midlife metabolic diseases that confer increased risk for late-onset AD (e.g. diabetes, obesity). Previously, our lab published work showing that four weeks of exogenous irisin treatment prevented the emergence of hippocampal pathology in female htau mice, a transgenic model of AD-like tauopathy. Here, we present data expanding this work that a) investigated irisin’s effects in 3xtg mice that develop both amyloid and tau pathology, and b) identified specific cytokines altered by irisin treatment in htau mice that

support its anti-inflammatory effects in the hippocampus. Adding further complexity to irisin's potential, we identified sex-divergent effects in irisin treatment-response in both AD mouse models. Our results further support irisin's potential as a neuroprotectant against AD-related etiology and emphasize the importance of identifying critical sex differences in treatment response.

Disclosures: K.A. Bretland: None. **K.M. Bretland:** None. **L. Lin:** None. **S. Terrill:** None. **G. Leone:** None. **C.M. Dengler-Crish:** None.

Poster

P238. Therapeutic Strategies: Preclinical Animal Models and Peptide-Based Therapeutics

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P238.08

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

Support: NINDS Grant R01NS28785

Title: Kifc1, a microtubule-associated motor protein, provides a novel avenue towards ameliorating synapse loss during neurodegenerative disease

Authors: *S. GUHA, H. MURALIDHARAN, P. W. BAAS;
Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Microtubules serve as tracks along which cellular organelles are transported in the axon by molecular motor proteins. Certain motor proteins also organize the microtubules themselves. We are exploring one such kinesin, namely KIFC1, which is a member of the kinesin-14 sub-family. Also called HSET in humans, KIFC1 has been studied extensively as a potential oncotarget due to its upregulation of expression in various cancers. However, inhibition of KIFC1 as a cancer therapy comes with potential deleterious side-effects due to its role in germ cell development and, as suggested by our previous work, might imperil axonal microtubule organization. Here we show that interactions of KIFC1 with the plus end of the microtubule and membrane proteins are necessary for the maintenance of synaptic connections. Specifically, we show that pharmacological inhibition of KIFC1 leads to a reduction in the number of synapses and dendritic spines. Given that synapse loss is a hallmark of many neurodegenerative diseases resulting in cognitive as well as sensory and motor impairments, we wondered if KIFC1 dysregulation may be a common downstream effector of synapse loss during such diseases. Oxidative stress often serves as a prelude to synaptic loss brought on by the release from surrounding activated astrocytes of molecules such as NO (nitric oxide) and ROS (reactive oxygen species). Our data show that in conditions of oxidative stress, KIFC1 levels are indeed diminished at the synapse. On this basis, we propose that fortifying KIFC1 in axonal terminals

and dendrites may provide a novel avenue toward ameliorating synapse loss in a variety of neurodegenerative diseases.

Disclosures: S. Guha: None. **H. Muralidharan:** None. **P.W. Baas:** None.

Poster

P242. Frontotemporal, Vascular, and Other Non-Alzheimer Dementias II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P242.01

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

Support: NIA P30 AG062566
R01 AG062566
U01 AG016976
R01 NS085770
NIA K08AG065463
NIH 5T32NS047987-14

Title: Contributions of Tau and TDP-43 Hippocampal Pathology in Amnestic Dementia

Authors: *G. MINOGUE[1], *G. MINOGUE[2], A. KAWLES[2], N. GILL[2,3], M. KOCHHEISER[2], R. KESZYCKI[2,4], V. LUBBAT[2], K. AJROUD[2,5], C. SPENCER[2], J. LILEK[2,5], Q. MAO[2,5], M. E. FLANAGAN[2,5], H. ZHANG[2,3], M.-. M. MESULAM[2,6], C. GEULA[2,7], T. GEFEN[2,4];
[1]Northwestern Univ., Chicago, IL; [2]Mesulam Ctr. of Cognitive Neurol. and Alzheimer's Dis., Feinberg Sch. of Med. at Northwestern Univ., Chicago, IL; [3]Dept. of Preventive Med., [4]Dept. of Psychiatry and Behavioral Sci., [5]Dept. of Pathology, [6]Dept. of Neurol., [7]Dept. of Cell and Developmental Biol., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: Introduction. Dementia syndromes are often not related to a single pathophysiologic process, leading to multiple neuropathologies found at autopsy. For example, an amnestic dementia syndrome can be associated with comorbid phosphorylated transactive response DNA-binding protein 43 (TDP-43) and Alzheimer's disease (AD). These pathologies are typically observed in hippocampal regions (mainly the CA1 sector) early in disease course. Those with comorbid AD and TDP-43 pathology ("AD/TDP") show worse long-term recall abilities compared to those with amnestic dementia due to AD. Here, we investigated neuronal integrity, pathologic burden, and hemispheric distributions of TDP-43 inclusions and neurofibrillary tau-positive tangles (NFTs) in the hippocampus, and its CA1 sector, in amnestic dementia due to AD/TDP and due to AD alone.
Participants and Methods. Twelve individuals with AD/TDP [mean age at death=81.31 (SD=11.14)], and 12 individuals with AD [mean age at death = 81.85 (SD=9.3)] were identified from the Northwestern AD Center brain bank. Cases carried an antemortem diagnosis of

amnestic dementia and met criteria for high AD neuropathologic change; AD/TDP were positive for at least medial temporal TDP-43. Left and right hippocampi were stained immunohistochemically with phosphorylated TDP-43 and AT-8 to visualize TDP-43 inclusions and NFTs, respectively. The HALO software (Indica Labs) was used to generate number of inclusions per mm^2 and % area occupied by immunopositivity in hippocampus and its CA1 sector. A linear regression was used to determine group differences with disease duration and APOE-4 allele status (0,1,2) included as covariates.
Results. There was no difference between the AD and AD/TDP groups in amount of NFTs or neuronal counts; however, compared to the AD group, the AD/TDP group showed significantly higher counts of *total* inclusions (NFTs + TDP-positive) after controlling for neuronal density in the CA1 sector (p=0.008). Distributions of pathology in CA1 and hippocampus were symmetric (ie., L=R) in both groups. Within the AD/TDP group, there was significantly more tau positivity (% area covered) compared to TDP-43-positivity in CA1 (p=0.036), but not the hippocampus in entirety.
Conclusions. The contribution of TDP-43 inclusions within the hippocampus to the amnestic phenotype appears to be additive, and perhaps represents a distinct pathophysiologic process from Alzheimer tau pathogenesis. Future studies will address questions of clinicopathologic concordance between markers of neurodegeneration and cognition during life.

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Poster

P242. Frontotemporal, Vascular, and Other Non-Alzheimer Dementias II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P242.02

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

Support: NIH Grant 1R01NS093362-01
NIH Grant 3R01NS093362-04S1

Title: Proximity-based labeling reveals DNA damage-induced N-terminal phosphorylation of fused in sarcoma (FUS) leads to distinct changes in the FUS protein interactome

Authors: *M. JOHNSON, T. A. NUCKOLS, P. MERINO, P. BAGCHI, S. NANDY, J. ROOT, G. TAYLOR, N. T. SEYFRIED, T. KUKAR;
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Abstract: Fused in sarcoma (FUS) is an RNA/DNA binding protein that participates in both nuclear and cytoplasmic functions. However, accumulation of FUS in the cytoplasm can lead to the formation of pathologic inclusions related to two neurodegenerative disorders, frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS). While most ALS-FUS cases are caused by pathogenic mutations in *FUS*, the vast majority of FTLD-FUS cases are not caused by *FUS* mutations. Therefore, identification of non-genetic mechanisms that cause FUS to accumulate in the cytoplasm is crucial to understanding FTLD and ALS pathogenesis. To this end, DNA damage can trigger the DNA dependent protein kinase (DNA-PK) to phosphorylate FUS at N-terminal residues leading to FUS accumulation in the cytoplasm. However, the functional consequences of FUS phosphorylation are unknown. Proximity labeling approaches paired with mass spectrometry (MS) allow identification of a target protein's interactome by labeling its potential interacting partners. In this study, we performed proximity-dependent biotin labeling via ascorbate peroxidase 2 (APEX2) paired with MS to investigate whether phosphorylation shifts the FUS interactome and protein function. We mapped the interactome of wild-type FUS, phosphomimetic FUS (a proxy for phosphorylated FUS), and P525L FUS (a pathogenic mutation that causes ALS). We demonstrate that expression of phosphomimetic FUS shifts the FUS interactome toward more cytoplasmic functions including mediation of mRNA metabolism and translation. Our findings reveal that phosphorylation of FUS may disrupt homeostatic translation and steady state levels of certain mRNA transcripts. These results highlight the importance of phosphorylation as a modulator of FUS interactions and functions with a potential link to disease pathogenesis. Data are available via ProteomeXchange with identifier PXD026578.

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Poster

P242. Frontotemporal, Vascular, and Other Non-Alzheimer Dementias II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P242.03

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

Support: DFG Grant 454245528

Title: Investigating the pathological crosstalk between cerebral amyloid angiopathy and arteriolosclerosis

Authors: *V. PEROSA[1], M. ZANON ZOTIN[2], C. A. AUGER[3], S. GREENBERG[1], A. VISWANATHAN[1], S. J. VAN VELUW[1,4];
[1]Neurol., [2]JPK Stroke Res. Center, Massachusetts Gen. Hosp., Boston, MA; [3]3MassGeneral Inst.

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Abstract: Cerebral small vessel disease (CSVD) is the leading cause of haemorrhagic stroke and a contributing pathology to dementia[1,2]. The most common subtypes of CSVD are cerebral amyloid angiopathy (CAA), characterized by the thickening of the walls of leptomeningeal and cortical vessels and loss of smooth muscle cells, as a result of amyloid-β (Aβ) accumulation, and arteriolosclerosis, which mainly affects the white matter vessels and is associated with hypertension. It remains poorly understood to what extent these pathologies overlap[3] and how they contribute to the development of different types of microvascular injury. We aimed to address these knowledge gaps in a cohort of 19(74.1±8.2y, 7F) autopsy cases with a clinical diagnosis of probable CAA.Formalin-fixed intact hemispheres were scanned on a 3T MRI scanner, including a 500μm^3 T2-weighted sequence, on which cortical microinfarcts (CMIs) and cerebral microbleeds (CMBs) were counted[4] and MRI-visible perivascular spaces (PVS) in the centrum semiovale were rated. Arteriolosclerosis was assessed on hematoxylin&eosin (H&E) stained sections in the white matter of four pre-defined juxtacortical areas using a semiquantitative score[5]. Furthermore, the area occupied by enlarged PVS (EPVS) was calculated on ImageJ with an in-house developed tool. Deep learning-based models were used to quantify leptomeningeal and cortical CAA, as previously described[6].All CMBs were located in the cortex, as typically seen in CAA, whereas no CMBs were found in subcortical locations. Cumulative score of arteriolosclerosis severity and CAA percentage area were not associated with total number of CMIs, CMBs, and EPVS severity on ex vivo MRI (Spearman's ρ_{CMIs}=-0.49, p=0.842; ρ_{MBs}=-0.18, p=0.450; ρ_{EPVS}=-0.35, p=0.138). Instead, bothtotal CAA percentage area and arteriolosclerosis were a predictor of EPVS percentage area on histopathology (no. of sections = 71; arteriolosclerosis: β=0.34, p<0.001; total CAA percentage area: β=0.34, p=0.014). CAA percentage area was not associated with arteriolosclerosis severity (no. of sections=71; total CAA: β=0.02, p=0.787).These preliminary findings suggest that the severity of arteriolosclerosis is not directly related to CAA burden in the overlying cortex in patients with a clinical diagnosis of CAA. However, these pathologies frequently co-exist and appear to both contribute to the burden of EPVS, a potential indicator of impaired paravascular clearance. Future directions include understanding how the burden and distribution of arteriolosclerosis (and other co-existing pathologies)contribute to creating a spectrum of clinical phenotypes within CAA.

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Poster

P242. Frontotemporal, Vascular, and Other Non-Alzheimer Dementias II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P242.04

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

Support: NIH Grant R01NS105971
NIH Grant R01NS093362
The Bluefield Project to Cure Frontotemporal Dementia
BrightFocus Foundation

Title: Investigating the ability of granulin subunits to rescue lysosome dysfunction, inflammation, and neurodegeneration in a mouse model of Frontotemporal Dementia

Authors: *J. ROOT[1,2], M. WANG[2], G. TAYLOR[2], T. L. KUKAR[2];
[1]Emory Univ. Sch. of Med. Neurosci. Grad. Program, Atlanta, GA; [2]Pharmacol., Emory Univ., Atlanta, GA

Abstract: Frontotemporal dementia (FTD) is the second most common dementia diagnosis after Alzheimer's disease. Heterozygous, loss- of-function mutations in the granulin gene (*GRN)* cause FTD by reducing the production of the progranulin (PGRN) protein. These genetic discoveries demonstrate that PGRN is important for neuronal health, but it is unclear why the loss of PGRN causes FTD. Interestingly, homozygous *GRN* mutations cause neuronal ceroid lipofuscinosis (NCL; *CLN11*), a lysosomal storage disorder (LSD) defined by dementia, lipofuscinosis, and neurodegeneration. The discovery that homozygous *GRN* mutations cause an LSD and that FTD-*GRN* patients share pathological features of NCL, strongly suggests lysosomal dysfunction underlies all PGRN deficient disorders, including FTD. The Kukar lab discovered that PGRN is rapidly processed into stable granulins in the lysosome, supporting the idea granulins have a beneficial function in the lysosome (Holler et al, *eNeuro*, 2017). Other groups have found a lysosomal origin of granulins and a beneficial function in multiple model systems. In contrast, some reports have suggested granulins are pro-inflammatory and could be neurotoxic. To help resolve these conflicting results, we designed a study to compare the ability of progranulin and granulins to ameliorate FTD-like phenotypes in a mouse model of PGRN deficiency. Here we report *in vivo* efficacy studies testing the hypothesis that delivery and replacement of a single granulin protein (granulin-2) to the brains of $Grn^{-/-}$ mice is sufficient to ameliorate neuroinflammation and neuropathology. This was accomplished by injection of various recombinant adeno-associated virus (rAAV2/1) into the cerebral ventricles of neonatal $Grn^{-/-}$ mice. Cohorts of $Grn^{-/-}$ and wild-type (WT) mice were injected with rAAV2/1 encoding granulin-2, full-length PGRN (positive control for delivery of all GRNs), or GFP (negative control). Following injections, mice were aged for 12 months, and tissues were harvested. We are now assessing biochemical and histopathologic markers of lysosomal dysfunction, neuroinflammation, and neurodegeneration. Galectin-3 (LGALS3), LAMP1, lipofuscin, Iba1, GPNMB, NeuN, and p62 will be measured via immunohistochemical staining, ELISA, or immunoblot. Using these measures, we will evaluate the extent to which an individual granulin subunit can rescue FTD-like phenotypes in aged $Grn^{-/-}$ mice. This study will provide clarity whether granulins are beneficial to neuronal health, and provide important insight into whether single granulin subunits may serve as effective therapeutics to treat diseases caused by PGRN deficiency.

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Poster

P242. Frontotemporal, Vascular, and Other Non-Alzheimer Dementias II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

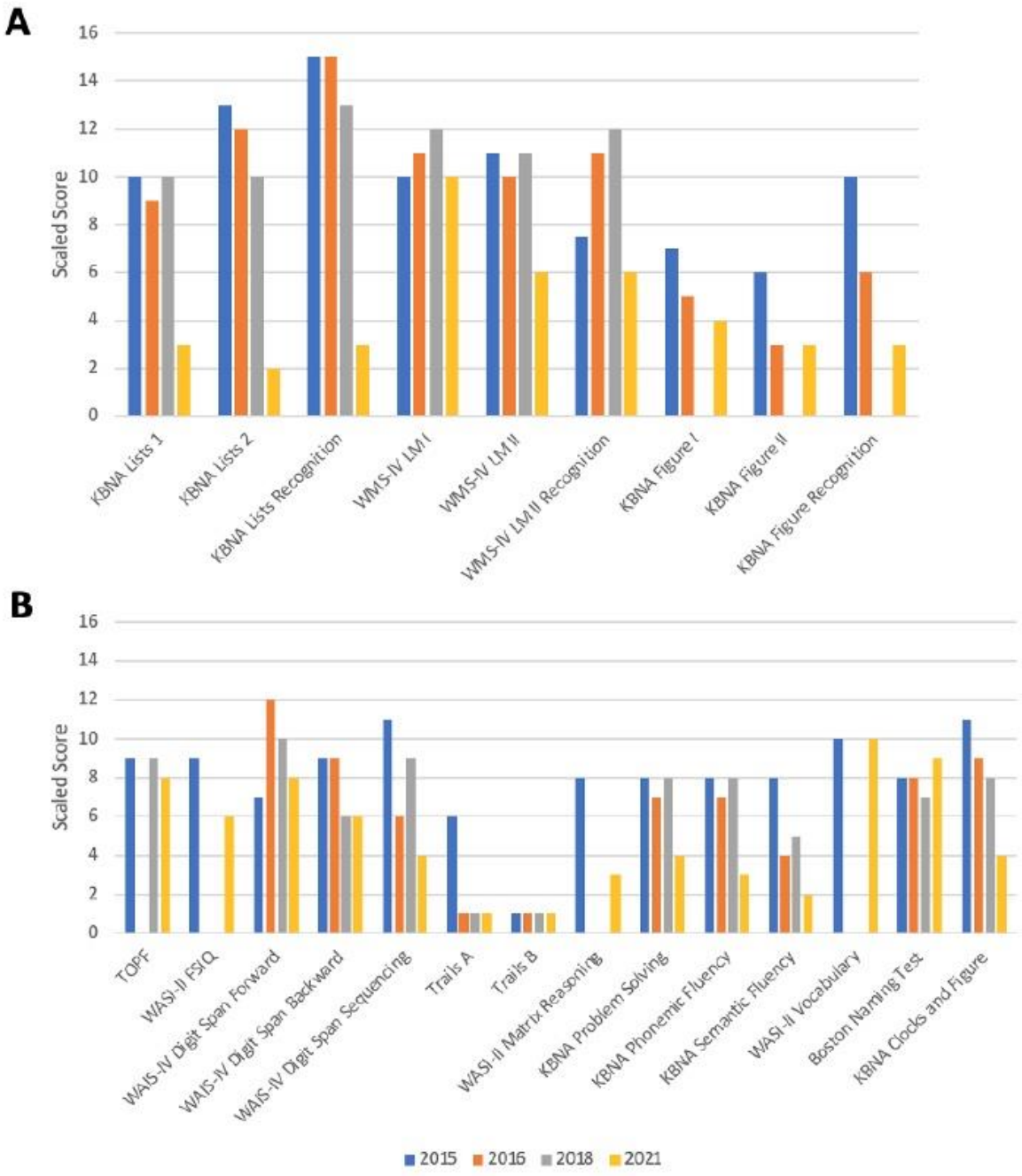
Program #/Poster #: P242.06

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

Title: Conversion to major neurocognitive disorder after COVID-19 in a woman with bipolar disorder: A 6-year longitudinal case report.

Authors: *E. TOLSDORF[1,2], C. GOJMERAC[2,3], J. CROWSON[2,3], B. FREY[2,4], F. KAPCZINSKI[2,5], D. DUARTE[2,3];
[1]McMaster Univ. Neurosci. Grad. Program - Minds, Hamilton, ON, Canada; [2]Psychiatry and Behavioural Neurosciences, McMaster Univ., Hamilton, ON, Canada; [3]Seniors Mental Hlth. Program, [4]Mood Disorders Program, St. Joseph's Healthcare, Hamilton, ON, Canada; [5]Inst. Nacional de Ciência e Tecnologia Translacional em Medicina (INCT-TM), Porto Alegre, Brazil

Abstract: It has been widely reported that the effects of SARS-CoV-2 infection tend to affect certain groups of individuals disproportionately, such as for the elderly and those with existing severe mental disorders. Recent research has shown increased COVID-19 symptom severity for individuals with dementia, although there are no reports showing an association between COVID-19 and the conversion from mild to major cognitive impairment. We present the 6-year longitudinal case of a woman with bipolar disorder who develops major vascular neurocognitive impairment following SARS-CoV-2 infection. This patient presented stable mild cognitive impairment for six years prior to switching to major cognitive impairment following positivity for SARS-CoV-2 complicated by a urinary tract infection. We discuss the cognitive and neuroanatomical repercussion prior to and following the infection of SARS-CoV-2 for this patient. We highlight that patients with low-grade inflammation due to severe mental health conditions may be uniquely susceptible to worse outcomes if they become infected by SARS-CoV-2. Additionally, this report indicates that there may be a compounding effect of COVID-19 on top of existing neuroinflammation in bipolar patients which may result in accelerated neurodegeneration and, ultimately, the conversion to dementia.



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Poster

P242. Frontotemporal, Vascular, and Other Non-Alzheimer Dementias II

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Program #/Poster #: P242.07

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

Support: 5P20GM109098
P20GM103434
R56 NS117754-01

Title: Triple Transgenic Alzheimer's' Disease Mice Display Cerebrovascular Dysfunction, Compounded by Chronic Stress

Authors: *E. N. BURRAGE, T. COBLENTZ, S. PRABHU, J. FRAZIER, R. W. BRYNER, J. SIMPKINS, P. D. CHANTLER;
West Virginia Univ., Morgantown, WV

Abstract: Chronic stress is a powerful risk factor in the development and progression of countless diseases including dementia. Past work in our lab has shown that chronic stress induces cerebrovascular dysfunction and recently, impaired cerebrovascular hemodynamics has been identified as a preclinical marker of dementia pathology. Despite this, the compound impact of chronic stress on cerebrovascular function in an Alzheimer's disease model has yet to be explored. Here, we utilized a triple transgenic mouse model of Alzheimer's disease (3xTg AD) combined with our unpredicted chronic mild stress paradigm (UCMS), to examine middle cerebral artery (MCA) function and to test the hypothesis that chronic stress can accelerate vascular AD pathology. A subset of mice was euthanized immediately following 8 weeks of chronic stress while another subset of mice was euthanized 3 months post chronic stress. Following euthanasia, the MCA's were removed and positioned in a pressurized myobath. The MCA was exposed to increasing concentrations of acetylcholine (ACh; 10-9M to 10-4M), phenylephrine (PE; 10-9M to 10-4M), and sodium nitroprusside (SNP; 10-9M to 10-4M). Immediately following chronic stress exposure, MCA dilation to ACh in AD mice were impaired compared to wildtype control mice (6.2±1.9μm to 19.2±0.7μm, respectively; $p<0.05$). Wildtype mice that underwent UCMS showed similar response to ACh compared to AD control mice (7±2μm to 6.2±1.9μm, respectively). The combination of AD and UCMS resulted in further impaired MCA dilation to ACh compared to either AD control and wildtype UCMS mice (3±1.5μm vs 7±2μm, and 6.2±1.9μm, respectively; $p<0.05$). No differences were found between groups in the PE or SNP response. Interestingly, in the mice euthanized at 9 months of age, these trends persisted. Overall, these data suggest that AD induces similar cerebrovascular dysfunction as compared to chronic stress, with combination of these compounding the cerebrovascular dysfunction and accelerate vascular AD pathology, irrespective of age of euthanasia.

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Poster

P242. Frontotemporal, Vascular, and Other Non-Alzheimer Dementias II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P242.08

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

Support: NIH R01 AG031867
Washington University Center of Regenerative Medicine Postdoctoral Fellowship

Title: Valosin-containing protein and the regulation of TDP-43 aggregation in neurodegeneration and myopathy

Authors: *E. LYNCH, S. PITTMAN, J. DAW, C. C. WEIHL;
Neurol., Washington Univ. Sch. of Med., Saint Louis, MO

Abstract: TDP-43 is a ubiquitously expressed RNA-binding protein commonly found aggregated in many neurodegenerative diseases and myopathies. TDP-43 is primarily localized in the nucleus but under conditions of cell stress will relocate to the cytoplasm as a component of stress granules. Under disease conditions aggregated TDP-43 remains in the cytoplasm and forms amyloid-like oligomers. These oligomers may initiate self-templated aggregate conversion of soluble TDP-43, leading to propagation and accumulation of phosphorylated insoluble aggregates. Dominant mutations in valosin-containing protein (VCP) cause several diseases which involve TDP-43 aggregation including frontotemporal dementia, inclusion body myopathy, and amyotrophic lateral sclerosis. In some cases such as multisystem proteinopathy (MSP), patients may have TDP-43 aggregation in both skeletal muscle and neurons. In these patients, the onset of myopathy often happens years earlier than the onset of dementia. Studies have shown that misfolded TDP-43 is capable of cell-to-cell transmission through neuronal networks, but it is unknown whether TDP-43 oligomers can spread from skeletal muscle to the central nervous system. We have two research goals: 1) to study TDP-43 aggregation in skeletal muscle to determine whether it is capable of self-templated conversion and spreading and 2) to investigate how mutations in *VCP* contribute to this process. Induced pluripotent stem cells (iPSCs) from VCP patients were differentiated into skeletal myocytes and analyzed for insoluble TDP-43 by fractionation western blot. We found that the VCP patient lines had increased insoluble TDP-43 compared to isogenic controls and this was further increased when treated with arsenite to induce cell stress. In addition, C2C12 mouse myoblasts which were treated with arsenite followed by a VCP inhibitor had a persistence of insoluble TDP-43 compared to arsenite-only controls. Together, these results suggest that mutations in *VCP* may facilitate TDP-43 aggregation as previously suggested, or lead to decreased clearance. A TDP-43 fluorescence resonance energy transfer (FRET) sensor line will be used to determine whether the muscle-derived TDP-43 aggregates are capable of seeding further aggregation. In addition, we used a tetracycline-activated expression system to generate a transgenic mouse line which expresses human TDP-43 with a mutated nuclear localization signal in skeletal muscle (rtTA-CKM x tetO-hTDP-43_{NLSm}). We plan to use these mice to further study mechanisms of TDP-43 aggregation in skeletal muscle and whether it is capable of propagation.

Disclosures: E. Lynch: None. **S. Pittman:** None. **J. Daw:** None. **C.C. Weihl:** None.

Poster

P242. Frontotemporal, Vascular, and Other Non-Alzheimer Dementias II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P242.09

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

Support: National Institute of Neurological Disorders and Stroke (NS085770)
Alzheimer's Disease Center Grant from the National Institute on Aging (AG013854)

Title: Increased Accumulation of Synaptic Proteins in Microglia Suggests Enhanced Synaptic Pruning in Frontotemporal Lobar Degeneration with TDP-43 Proteinopathy

Authors: *I. A. AYALA, C. SPENCER, M. E. FLANAGAN, T. D. GEFEN, M. MESULAM, C. GEULA;
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Abstract: Frontotemporal lobar degeneration (FTLD) is characterized by degeneration of the frontal and temporal lobes and abnormalities in behavior and/or language. Synaptic loss is among the first changes associated with cognitive decline in aging and Alzheimer's disease (AD). Aberrant synaptic pruning by microglia may contribute to loss of synapses in AD. We have observed decreased cortical levels of synaptic proteins in FTLD with TDP-43 pathology (FTLD-TDP). In this study we investigated accumulation of synaptic proteins in microglia as a surrogate marker for potential increased synaptic pruning in behavioral variant frontotemporal dementia with TDP-43 pathology. Tissue from middle frontal gyrus of seven participants was used (Control, n=3, FTLD-TDP, n=4). Paraffin-embedded sections underwent double immunofluorescent processing to concurrently visualize microglia and synaptic proteins. Specific antibodies to the presynaptic protein synaptophysin, the postsynaptic protein PSD-95, and the dendritic spine protein spinophilin were used to visualize immunoreactivity for each synaptic protein. Antibodies to Iba1 or HLA-DR were used to visualize microglia. Reagents were chosen to fulfill the requirement of double immunofluorescent staining that the two antibodies are raised in different species. Modified unbiased stereological methods were used for quantitative analysis. Counts were obtained from three types of synaptic staining in microglia: 1) microglia with no synaptic proteins, 2) microglia with punctate synaptic proteins, and 3) microglia with large aggregates of synaptic proteins. The three patterns were observed in all cases and synaptic proteins. Significantly larger numbers of microglia displayed aggregates of PSD-95 than punctate staining in FTLD-TDP participants ($p<0.05$), whereas there were no significant differences between these two patterns in control participants ($p>0.05$). Furthermore, the percentage of total microglia that contained aggregates of synaptophysin was significantly

larger in FTLD-TDP when compared with controls ($p<0.03$). The number of microglia containing aggregates of spinophilin was significantly higher than microglia containing punctate staining in both FTLD-TDP and controls ($p<0.02$). These results point to accumulation of synaptic proteins in microglia, with larger aggregates predominating for some synaptic proteins in FTLD-TDP. One potential interpretation of these results is increased synaptic pruning. Mechanistic studies in animal models are required to determine whether aberrant synaptic pruning is indeed a feature of TDP-43 proteinopathies.

Disclosures: I.A. Ayala: None. **C. Spencer:** None. **M.E. Flanagan:** None. **T.D. Gefen:** None. **M. Mesulam:** None. **C. Geula:** None.

Poster

P279. Ataxia and Dystonia: Models

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P279.01

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Grant R21-HD097557
NIH Grant RO1-GM120358

Title: Defective myelination in an RNA polymerase III mutant leukodystrophic mouse

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Abstract: Leukodystrophies constitute a group of genetically heterogeneous inherited diseases that affect myelin sheath production and/or function in the central nervous system (CNS). RNA polymerase (Pol) III-related leukodystrophy, an autosomal recessive disorder, is the second most prevalent leukodystrophy and was identified when five initially distinct syndromes with overlapping clinical and radiological features were found to have a common molecular basis. Patients are diagnosed between birth and adolescence with neurological and non-neurological deficits and are classified according to distinct brain MRI features and confirmed by sequencing. RNA polymerase (Pol) Pol III-related leukodystrophies involve mutations primarily in *Polr3a* and *Polr3b*, which encode the two largest subunits and form the catalytic core of the enzyme. Pol III synthesizes abundant short non-coding RNAs that have essential functions in protein synthesis, secretion and other processes. Despite the ubiquitous functions of these RNAs, the basis of this neural sensitivity and the mechanisms of disease pathogenesis remain unknown. We show that conditional knock-in of pathogenic *Polr3a* mutations in the Olig2 lineage in mice

results in growth, neurobehavioral and hypomyelination phenotypes in multiple regions of the cerebrum and spinal cord reflecting a subset of clinical features found in patients. In contrast, the gross motor defects and cerebellar hypomyelination that are common features of severely affected patients are absent in the mice, suggesting a relatively mild form of the disease in this conditional model. Our results show that disease pathogenesis in the mice involves defects that reduce both the number of mature myelinating oligodendrocytes and the ability of these cells to produce a myelin sheath of normal thickness. Our findings uncover a critical role for Olig2 expressing cells in disease pathogenesis, specifically in the development and/or survival of oligodendrocytes as well as their function in myelination.

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Poster

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Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: Quadrant Biosciences, Inc.

Title: Functional consequences of novel mutations in the PANK2 gene identified in a large Dominican Republic cohort with pantothenate kinase associated neurodegeneration (PKAN)

Authors: I. OJUKWU[1], C. MUNIZ[2], S. BASER[3], C. BASS[4], M. SANTANA[5], *F. MIDDLETON[2];
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Abstract: Pantothenate Kinase Associated Neurodegeneration (PKAN) is an autosomal recessive genetic disorder caused by mutations in the gene encoding PANK2 (Pantothenate Kinase 2) an enzyme essential for vitamin B5 metabolism highly expressed in the basal ganglia. PKAN typically begins in late childhood causing dystonia, bradykinesia, dysarthria, tremors, and muscle wasting, although the pattern and severity vary between subjects. While rare globally (affecting 1 in 1,000,000 people worldwide) PKAN is common in the southwestern Dominican Republic (DR), where it affects more than 1 in 1,000 persons and where we have done extensive studies to gain insight into disease mechanisms and symptom heterogeneity. Comprehensive genotyping and whole exome analyses indicate that most affected DR subjects are homozygous for a Y227C mutation in the PANK2 protein unique to the DR. However, we also recently identified a novel R532W mutation in PANK2 protein transmitted in a bilineal compound

heterozygous fashion to 3 affected siblings. Whole exome sequencing and genotypic data analysis failed to reveal any other mutations in PANK2 or genes associated with other movement disorders, including NBIA, dystonia, Parkinson's disease, Huntington's disease or Wilson's disease. We hypothesized that both the Y227C and R532W mutations have reduced phosphorylation activity and disrupted protein-protein interactions that lead to neurodegeneration. To test this, we first performed in silico modeling of the potential effects of each mutation on protein conformation. This consistently predicted deleterious effects of the Y227C mutation in chain C of the PANK2 protein homotetramer due to conformational changes and disrupted binding interactions, but less consistent predictions regarding the effects of the R532W mutation. Consequently, we generated expression vectors for the wildtype (WT) human PANK2 gene as well as two single-mutant (Y227C and R532W) forms of the gene, to express His-tagged versions of each full-length protein. Expressed proteins were affinity purified and used for biochemical studies to monitor rates of end product formation by reverse-phase LC-MS. We also visualized the potential differences in enzyme activity and compared the cellular localization of the WT and mutant enzymes using Click-It Chemistry and immunofluorescent microscopy. Co-immunoprecipitation and MS analysis are currently in progress to reveal potential differences in protein-protein binding partners. Finally, cellular viability and transcriptional responses are being compared to gain additional insight into the impact of these specific mutations compared with others in the PKAN literature.

Disclosures: I. Ojukwu: None. **C. Muniz:** None. **S. Baser:** None. **C. Bass:** None. **M. Santana:** None. **F. Middleton:** None.

Poster

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Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NINDS Grant R01NS050808
NINDS Grant R01NS105470

Title: Cerebellar mGluR1 receptors are required for caffeine-induced attacks of dyskinesia in Episodic Ataxia Type 2

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Abstract: Episodic Ataxia type 2 (EA2), is caused by mutations in the CACNA1A gene, which encodes the pore forming subunit of the P/Q type voltage gated calcium channel. These

mutations result in a loss-of-function of the P/Q type calcium channel, which leads to baseline ataxia, and attacks of dyskinesia, that can last a few hours to a few days. These attacks are brought on by consumption of caffeine, ethanol, and physical or emotional stress. Using the *tottering* mouse, a well characterized mouse model of EA2, we have previously established that stress induces attacks by activation of cerebellar α1 adrenergic receptors. Activation of these receptors leads to an increase in casein kinase 2 (CK2) mediated phosphorylation of the calmodulin associated with SK channels, which decreases SK channel activity, causing an increase in Purkinje cell irregularity and attacks of dyskinesia. Because caffeine can increase NE levels and change the expression of α1 adrenergic receptors, we explored whether it also induced attacks by the same mechanism. We found that caffeine increased phosphorylated calmodulin in the cerebellum, suggesting that caffeine may also induce attacks via a CK2 dependent signaling pathways. Similar to stress, both caffeine-induced attacks and Purkinje cell firing irregularity were prevented by shRNA knock down, or block, of CK2. Block of α1 adrenergic receptors, however, failed to prevent caffeine induced attacks. Since at the concentrations that it causes attacks in EA2 caffeine is known to block A1 adenosine receptors, we explored a potential role for this pathway. We found that block of A1 adenosine receptors alone, induced attacks of dyskinesia and increased Purkinje cell firing irregularity to a similar extent as caffeine, suggesting caffeine indeed induces attacks through this mechanism. Block of A1 adenosine receptors increases the release of neurotransmitters such as glutamate, thus we hypothesized that activation of mGluR1 receptors on Purkinje cells led to the increased irregularity and attacks of dyskinesia in EA2. We found activating mGluR1 receptors induced attacks of dyskinesia, and the block of mGluR1 receptors significantly decreased the percent of caffeine-induced attacks. Finally, we discovered a novel direct interaction between mGluR1 and CK2, and the inhibition of mGluR1 prior to initiation of attack prevented the caffeine-induced increase in phosphorylated calmodulin. Given the success of pharmacological block of CK2 to prevent stress and caffeine induced attacks, we believe these data establish the ground-work for the development of therapeutics for the treatment of caffeine and stress induced attacks in EA2 patients.

Disclosures: H. Snell: None. **A. Vitenzon:** None. **K. Khodakhah:** None.

Poster

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Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: National Ataxia Foundation
National Institutes of Health

Title: Endogenous tagging of RAN peptides in Fragile X-associated Tremor/Ataxia Syndrome.

Authors: *G. SKARIAH, C. J. MALTBY, A. KRANS, P. K. TODD;
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Abstract: Fragile X-associated Tremor/Ataxia Syndrome (FXTAS) is caused by the expansion of a CGG trinucleotide repeat to a pre-mutation range (55-200 repeats) in the 5'UTR of the FMR1 gene. CGG repeat expansions in FXTAS support translation of homopolymeric proteins through a process known as CGG Repeat-associated non-AUG Translation (CGG RAN). Generation of the most abundant CGG RAN product, a polyglycine containing protein called FMRpolyG, elicits toxicity in human cells, Drosophila and transgenic mouse models of FXTAS. Accurate detection, tracking and quantification of RAN translation products from endogenous gene loci would markedly expand our ability to determine how they elicit dysfunction and to confirm selective inhibition of their production as a first step towards therapy. Unfortunately, currently available antibodies that readily detect heterologous expression of these proteins are not reliably effective at staining the endogenous untagged RAN products in a fashion that readily allows for their quantification. To address this, we designed specific CRISPR-Cas9 guides to incorporate either a luminescence based HiBiT-LgBiT tag or a split mNeonGreen21-10/11 (mNG2) fluorescent tag in the FMR1 locus in FXTAS patient derived induced pluripotent stem cells (iPSCs). I tested the CRISPR reagents in HEK cells by tagging the endogenous FMR1 locus in the FMRpolyG frame. We observed a modest increase in HiBiT signal in the setting of a normal repeat in HEK cells suggesting very low endogenous expression of FMRpolyG. Plasmid-based overexpression studies in HEK cells confirmed that the HiBiT tag expresses even in the context of expanded CGG and C9 repeats. In addition, we have successfully incorporated FLAG tags in the same locus to facilitate immunoprecipitation of the FMRpolyG RAN peptide. We are now optimizing the mNG2 system in FXTAS and iPSC lines to detect FMRpolyG in human patient neurons. Once established, we will validate these tools using a series of recently identified RAN translation specific modifiers.

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Poster

P279. Ataxia and Dystonia: Models

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Program #/Poster #: P279.05

Topic: C.04. Movement Disorders other than Parkinson's Disease

Title: Spherical Nucleic Acids Targeting FXN Induce Frataxin Expression in Models of Friedreich's Ataxia

Authors: *G. T. CORBETT[1], U. A. BARAY[1], S. ANANTATMULA[1], A. K. LAGRIMAS[1], N. CRAMB[1], H. GURLER-MAIN[1], G. BALANGUE[1], A. EINHORN[1], S. MIX[1], E. W. BUSS[1], L. R. MOORE[1], A. SCHOOK[1], C. R. MURATORE[2], L. P. KELLY[1], W. L. DANIEL[1], M.

SCHROFF[1], B. R. ANDERSON[1];
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Abstract: Friedreich's Ataxia is a progressive neurodegenerative disorder caused by mutations in the *FXN* gene. Expanded intronic GAA repeats in *FXN* result in downregulation of frataxin protein, mitochondrial dysfunction, and neurodegeneration. Currently, there are no approved therapies to slow or prevent progression of FA. Spherical nucleic acids (SNA^{TM}) are nanostructures comprised of densely packed oligonucleotides radially arranged around a nanoparticle core. Compared to their linear counterparts, SNA show increased cellular uptake and persistence in the CNS. In this study, we designed and assessed several mono-targeting and multi-targeting FXN-SNA that exert two mechanisms of action for upregulation of *FXN* mRNA: i) transcript stabilization and ii) FXN natural antisense transcript (FXN_{NAT}) degradation. FXN-SNA were assessed for efficacy in FA patient fibroblasts and in induced neurons (iNs) generated from FA patient-derived induced pluripotent stem cells. In the absence of transfection agents, FXN-SNA exhibited strong FXN mRNA upregulation in patient cells. Importantly, FXN-SNA dose-dependently increased endogenous frataxin protein in the cytoplasm and mitochondria, and improved mitochondrial function in phenotypically-mature FA iNs. Several lead FXN-SNA were administered into the intra-cisterna magna space of mice expressing GAA-expanded human *FXN* in the absence of murine *Fxn* (*Fxn*^{null}::YG8s(GAA)$_{>800}$ mice). Fourteen days after dosing, brain, spinal cord and dorsal root ganglia (DRG) tissues were assessed for target engagement. FXN-SNA dose-dependently increased *FXN* mRNA and frataxin protein expression in the brain, spinal cord and DRG of humanized FA mice. Among the tested CNS regions, the strongest upregulation was observed in the cerebellum, a region critically affected in FA, where we observed up to 3-fold increases in frataxin protein versus vehicle. FXN-SNA also induced over a 2-fold increase in frataxin protein in dorsal root ganglia, another region greatly impacted in FA. Critically, we observed improvements in succinate dehydrogenase activity in these regions, a marker for mitochondrial function. All tested FXN-SNA were well-tolerated, as clinical and anatomic pathology assessments revealed no drug-related adverse events. In conclusion, we developed several FXN-SNA that exploit endogenous cellular machinery to upregulate FXN mRNA and protein via degradation of FXN_{NAT} and/or extension of *FXN* mRNA half-life. These findings support the continued development of FXN-SNA for potential treatment of FA and warrant exploration of SNAs for various other neurological diseases.

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Poster

P279. Ataxia and Dystonia: Models

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Program #/Poster #: P279.06

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: CIHR

Title: Dopamine Transporter Deficiency Syndrome mouse model shows reduced hyperactive phenotype in response to amphetamine and alhpa-methyl-para-tyrosine

Authors: *E. RUSSO[1], A. REZAYOF[1,2], P. BEEREPOOT[1], M. MILENKOVIC[1], D. E. WATKINS-CHOW[3], A. RAMSEY[1], A. SALAHPOUR[1];
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Abstract: Genetic mutations that result in misfolded proteins are a cause of a number of human diseases, one of which is Dopamine Transporter Deficiency Syndrome (DTDS). Autosomal recessive loss of function mutations in the dopamine transporter (DAT) lead to its retention and degradation in the endoplasmic reticulum (ER), ultimately resulting in low levels of functional DAT. A complex motor disorder arises, and patients diagnosed with DTDS display a classical clinical phenotype of initial hyperactivity that progresses over time to parkinsonism-dystonia. To gain additional information on the pathophysiology of this disease and to investigate potential pharmacotherapies we have created a new line of transgenic knock-in mice carrying one particular DTDS disease-causing DAT mutation. This mouse (mA313V) models the human DTDS mutation hA313V. Herein is a report on the initial behavioral and neurochemical characterization of the A313V mice as well as on promising drug treatments for the management of classic DTDS symptoms. A313V mice have approximately 5-fold less mature DAT in comparison to wildtype (WT) littermates and a consequent increase in locomotor activity as measured by the open field test. This hyperactivity can be reduced upon treatment of amphetamine and alpha-methyl-para-tyrosine (aMPT)

Disclosures: E. Russo: None. **A. Rezayof:** None. **P. Beerepoot:** None. **M. Milenkovic:** None. **D.E. Watkins-Chow:** None. **A. Ramsey:** None. **A. Salahpour:** None.

Poster

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Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH F31NS122495

Title: Novel ataxia phenotype due to spontaneous Myo5a mutation detected by whole genome sequencing

Authors: *A. M. TELENSON[1,2], R. R. HSIEH[1,2], A. H. VO[1,3], M. HADHAZY[1,2], L. PESCE[1], M. J. PUCKELWARTZ[1,2], E. M. MCNALLY[1,2];
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Abstract: Spontaneous mutations in mice contribute to understanding the genetic mechanisms of neurodegenerative diseases. A genetic backcross strategy was undertaken to assess the "Superhealing" MRL (Murphy Roths Large) strain for its ability to ameliorate the muscular dystrophy phenotype caused by the loss of the dystrophin associated protein gamma-sarcoglycan (*Sgcg*). In this context, during the fifth-generation backcross, a new spontaneous mutation was discovered and associated with a Novel Ataxic Phenotype (NAP). Breeding studies confirmed autosomal recessive inheritance, and genotyping confirmed the ataxic phenotype was separable from the *Sgcg* locus and thus the muscular dystrophy process. NAP mice first displayed ataxia by postnatal day 11, followed by defects in coordination, an abnormal righting reflex and hindlimb collapse. NAP mice exhibited rapid progression with severe motor deficits and lethality between days 21-25. Using Whole Genome Sequencing (WGS) on heterozygous parents, two affected offspring and an unaffected mouse from each of the original breeding lines, we applied systematic filtering of novel variants to uncover a single nucleotide polymorphism (SNP) in the 5' donor splice site of exon 14 of 41 in the *Myo5a* gene. *Myo5a* encodes an unconventional, processive actin-based myosin whose C-terminal tail engages with intracellular cargo. Autosomal recessive mutations in human *MYO5A* cause Griscelli Syndrome, a rare disorder associated with abnormal pigmentation, hypotonia and severe developmental delay. The splicing defect responsible for the findings in NAP mice show exon 14 exclusion, a significant reduction in *Myo5a* mRNA and a complete loss of myosin Va protein. *Myo5a* is highly expressed throughout the brain and in melanocytes, the latter contributing to coat color defects. Further neurological defects are being characterized, including the role of myosin Va in myelination and intracellular transport. Because the phenotype arose on the MRL background, we conclude that the Superhealing MRL background is insufficient to prevent the neuropathology in NAP mice.

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Poster

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Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIEHS Grant ES029344

Title: Slc39a14 knockout mice with increased blood and brain manganese levels and dystonia-parkinsonism exhibit increased TSPO levels in the cerebellum and brainstem indicative of active neurodegeneration

Authors: *A. N. RODICHKIN, J. L. MCGLOTHAN DZIEDZIC, T. R. GUILARTE;
Envrn. Hlth. Sci., Robert Stempel Col. of Publ. Hlth. & Social Work Florida Intl. Univ., Miami, FL

Abstract: In the last decade there has been an increasing number of reports describing individuals with inherited autosomal recessive mutations of the manganese (Mn) transporter gene *SLC39A14*. These individuals expressing the mutations have high concentrations of Mn in blood and brain and display childhood-onset dystonia-parkinsonism. Previously, we have reported that *Slc39a14*-knockout (KO) male and female young adult mice (postnatal day 60, PN60) exhibit increases in blood and brain Mn as well as dystonia-parkinsonism similar to the human cases. We have also reported that assessment of the *Slc39a14*-KO mice in nigrostriatal dopaminergic system showed no changes in striatal dopamine and metabolite concentrations or neurodegeneration of dopaminergic neurons of the substantia nigra pars compacta relative to wildtype (WT) mice. On the other hand, *Slc39a14*-KO mice have a marked inhibition of potassium-stimulated dopamine release in the striatum. To continue to characterize the pathophysiological changes in male and female PN60 *Slc39a14*-KO mice, we took a broad screening approach to assess ongoing neurodegeneration using quantitative Translocator protein 18 kDa (TSPO) autoradiography. TSPO is a well-validated quantitative biomarker of neuroinflammation resulting from brain injury and neurodegeneration that allows an unbiased screening approach to determine brain regions undergoing active degeneration. We used the TSPO specific radioligand [^{3}H]-DPA-713 to measure TSPO levels in multiple brain regions from wildtype (WT) and *Slc39a14*-KO male and female PN60 mice. Our results indicate no significant changes in [^{3}H]-DPA-713 specific binding to TSPO in several forebrain regions including cerebral cortex, striatum, globus pallidus, septal nucleus, hippocampus, amygdala, hypothalamus, and thalamus. However, we unexpectedly found significant increases in [^{3}H]-DPA-713 specific binding to TSPO in the cerebellum (male= 27.7%, female=16.7%), deep cerebellar nuclei (male=31.0%, female=21.1%), brainstem (male=29.1%, female=24.8%), and the facial motor nucleus of the brainstem (male=31.8%, female=22.2%) in *Slc39a14*-KO PN60 mice relative to WT. These results describe for the first-time active neurodegeneration in the cerebellum and brainstem of *Slc39a14*-KO mice, an observation that is consistent with the known role of these brain structures in the postural and gait instability in dystonia-parkinsonism.

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Poster

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Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: Don't Forget Morgan Foundation
NIH Grant P20GM103620
USD-N3 Fellowship DGE-1633213

Title: Wdr45 function and contribution to bpan

Authors: *B. L. MEYERINK, Z. C. STRICKLAND, J. J. BRUDVIG, J. M. WEIMER, L.-J. PILAZ;
Pediatrics and Rare Dis., Sanford Res., Sioux Falls, SD

Abstract: Mutations in the X-linked gene *WDR45* cause the rare disease Beta Propeller-protein Associated Neurodegeneration (BPAN) which leads to global developmental delay, intellectual disability, seizures, and premature death. *WDR45* encodes for a protein typically associated with autophagy regulators, though the protein's function and how its absence in BPAN patients leads to the disease is largely unknown. To explore protein function, we used a bait-BioID system to label protein interactors with WDR45 in neuroblastoma cells. We found a strong association of WDR45 with proteins involved in endomembrane-associated protein transport, RNA processing, and mitochondrial proteins important for metabolism of lipids and maintaining mitochondrial membrane potential. Defects in these pathways are known to occur in other neurodegenerative diseases such as Alzheimer's Disease, Parkinson's Disease, and Huntington's disease. To study WDR45's role in these pathways we have used iGONAD to make a mouse model with a genetically encoded epitope tag on endogenous WDR45 allowing specific detection of this molecule. This model will be used to explore WDR45's role in the pathways elucidated in the BioID screen and determine the expression pattern of WDR45 in the brain. We have also created a model introducing a patient-analogous premature stop codon early in the *WDR45* gene, ablating protein expression and more faithfully recapitulating the human disease phenotype in the mouse with early death occurring in homozygous mutant animals. We are characterizing this mouse line along with a previously generated BPAN model which has shown no altered survival phenotype in homozygous mutant animals. Our analysis focuses on early markers of neurodegeneration to characterize disease onset and progression. Knowledge of the neurodegenerative progression in these animals and causative mechanisms are key insights allowing for a more informed approach to therapeutic development. These findings reveal previously unexplored functions of WDR45 and potential causatives factors of BPAN pathology which may additionally give relevant insight to more common, phenotypically similar neurodegenerative diseases.

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Poster

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Program #/Poster #: P279.10

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: Dist. Scholar Award / University of Iowa

Title: Developing Adenoviral Vectors to target Distinct Cerebellar Cell Types

Authors: *E. KUL, S. PHILLIPS, P. VALINO-RAMOS, S. M. YOUNG, JR;
Dept. of Anat. and Cell Biol., Univ. of Iowa, Iowa City, IA

Abstract: Recombinant viral vectors have become integral tools for basic *in vivo* research applications. Helper-dependent adenoviral (HdAd) vectors are safe viral vectors with a large packaging capacity of ~36 kb of DNA and mediate long-term expression without toxicity. This carrying capacity of HdAd enables basic research and clinical fields requiring the delivery of large genes or multiple transgenes, which cannot be packaged into other widely used viral vectors, such as Adeno-associated virus (AAV). Genetic disorders involving the cerebellum, such as spinocerebellar ataxia type-6 (SCA6), ataxia-telangiectasia (A-T) and episodic ataxia type-2 (EA2) are caused by mutations in large genes and result in severe motor phenotypes in the affected individuals. Disfunction and degeneration of the Purkinje cell that is a key cell type of the cerebellum, is commonly observed in these disorders. Thus, successful delivery of large transgene constructs to Purkinje cells is essential in developing gene therapy approaches for these cerebellar disorders. Currently, the HdAd serotype 5 vectors are the most common adenoviral vector used in basic and clinical applications. However, HdAd type 5 vectors do not transduce Purkinje cells. To overcome the current limitations, we have been in the process of developing chimeric adenoviral vectors with altered viral tropisms. Here, we evaluate our vectors in the mouse cerebellum against molecular markers of distinct cerebellar cell types by colocalization studies. Furthermore, we discuss differential transduction patterns of each chimeric vector with a primary focus on the Purkinje cell.

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Poster

P291. Motor Neuron Disease: Animal Models I

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Topic: C.06. Neuromuscular Diseases

Support: Alzheimer's Association New Investigator Research Grant: NIRG-12-241456
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Delaware IDeA Network of Biomedical Research Excellence (INBRE) Pilot Award: NIH-NIGMS: 5P20GM103446
NIH-NIGMS Centers of Biomedical Research Excellence (COBRE): 5P20GM103653
Delaware Economic Development Office Grant from the State of Delaware

Title: Astrocyte-specific expression of triple mutant TDP-43 causes changes in memory and anxiety-like behavior

Authors: *S. MCGRIFF, M. GITCHO;
Delaware State Univ., Dover, DE

Abstract: TDP-43 is the major pathological protein in motor neuron disease, frontotemporal dementia and is also found in ~50% of those with Alzheimer's disease. Alexander disease is a developmental leukodystrophy and mutations in GFAP segregate with disease. In Alexander disease, pathological inclusions containing phosphorylated TDP-43 were discovered. Astrocyte pathology, though rare is present in all TDP-43 proteinopathies. We have developed a new mouse model to selectively drive human TDP-43 with three ALS familial mutations (3XTDP43) specifically in astrocytes (GFAP-tTA). Methods: Utilizing the tetracycline inducible system, we examined 12-month-old mice for behavior (rotarod, light/dark box, and Barnes maze), pathology, biochemistry, proteomics, and exon array. Our study seeks to understand the correlation between astrocyte driven pathological TDP-43 functional alters memory and anxiety-like behavior. Results show pathological changes in astrocytes with a decrease in glial fibrillary acidic protein (GFAP) expression. We further show changes in memory and anxiety-like behavior with little motor deficits. Therefore, astrocyte driven TDP-43 pathology and the development of functional changes associated with memory and anxiety may provide a better understanding of pathways associated with neurodegeneration in TDP-43 proteinopathies.

Disclosures: S. McGriff: None. **M. Gitcho:** None.

Poster

P291. Motor Neuron Disease: Animal Models I

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Program #/Poster #: P291.02

Topic: C.06. Neuromuscular Diseases

Support: National Institute on Aging 1K01AG042500
Delaware IDeA Network of Biomedical Research Excellence (INBRE) Pilot Award: NIH-NIGMS: 5P20GM103446
NIH-NIGMS Centers of Biomedical Research Excellence (COBRE) 5P20GM103653
Delaware Economic Development Office Grant from the State of Delaware

Title: Astrocyte-specific expression of tdp-43 during development causes blindness in 12-month-old mice

Authors: *W. SMITH, S. AREZOUMANDAN, M. GITCHO;
Delaware State Univ. Dept. of Biol. Sci., Dover, DE

Abstract: Transactive response DNA binding protein 43 (TDP-43) is the major pathological protein in motor neuron disease. In some models of TDP-43 overexpression, mice do not survive when the transgene is on throughout normal development. We utilized an inducible model with mutations in the nuclear localization signal that drives expression of TDP-43 in the cytoplasm (TDP-43ΔNLS) specifically in astrocytes. For this study, the transgene was turned on throughout their development (tet-off system). Methods: behavior, histology, biochemistry, and functional magnetic resonance imaging (fMRI) were evaluated 12 months of age. Results: At 12 months of age, the mice lost their ability to see, and the lens of the eyes became opaque and there was a complete disorganization of the photoreceptors. In addition, we used functional magnetic resonance imaging to evaluate the visual pathway of the mice in the 4 months of age. Our preliminary data indicate that astrocyte expression of TDP-43ΔNLS throughout their development causes changes in vision. These findings suggest that the mislocalization and alteration of expression in astrocytes of TDP-43 affects visual development in the mice.

Disclosures: W. Smith: None. **S. Arezoumandan:** None. **M. Gitcho:** None.

Poster

P291. Motor Neuron Disease: Animal Models I

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Program #/Poster #: P291.03

Topic: C.06. Neuromuscular Diseases

Support: Motor Neurone Disease (MND) Association UK (Miles/Apr18/863-791)
Chief Scientist Office (Scotland)

Title: Selective Vulnerability of Tripartite Synapses in Amyotrophic Lateral Sclerosis

Authors: *C. BONTHRON[1], M. J. BROADHEAD[1], J. WADDINGTON[1], M. LOPEZ[1], F. ZHU[2], S. G. N. GRANT[2], C. SMITH[2], G. MILES[1];

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Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder affecting the cortex, brainstem and spinal cord, resulting in progressive muscle paralysis. ALS has been described as a synaptopathy, with evidence of central and peripheral synaptic changes in animal models and patient post-mortem tissue. Astrocytes are key regulators of synaptic function and envelop synaptic terminals, forming 'tripartite synapses'. With growing evidence that astrocytes significantly contribute to ALS pathogenesis, here we aimed to investigate tripartite synapses in the context of disease. To do this, we performed high resolution quantitative microscopy of tripartite synapses in lumbar spinal cord sections obtained from $SOD1^{G93A}$ x PSD95-eGFP (Postsynaptic Density Protein 95) mice at several time points of disease progression, from presymptomatic stages through to the emergence of motor deficits (8 weeks [W], 12W, 16W). Tripartite synapses were visualised using PSD95-eGFP labelling in combination with immunohistochemical markers for presynaptic terminals (VGLUT2) and astrocyte contacts (EAAT2 and p-Ezrin). Stitched high magnification imaging of spinal hemisections allowed for the production of 'tripartite spinal maps', whereby Rexed laminae could be delineated allowing for region-specific analysis of millions of synapses from ALS mice. Analysis was conducted by multiple researchers blinded to animal genotype throughout. Overall, we observed progressive synaptic loss in 16W (early symptomatic) $SOD1^{G93A}$ mice. Interestingly, analysis of non-tripartite and tripartite synapses revealed selective decreases in **tripartite synapse density only**, when using either astrocytic marker (EAAT2: N=5, P<0.0001; p-Ezrin: N=4, P=0.0004). This loss of tripartite synapses was observed widely throughout spinal laminae. Furthermore, we observed the same selective vulnerability of tripartite synapses in human post-mortem tissue from ALS patients (N=6 control, N=9 ALS, P=0.011). These data suggest a selective vulnerability of tripartite synapses in ALS, providing further insight into previously reported synaptic loss which did not assess astrocytic components, and supporting a toxic role of astrocytic synaptic contacts in disease.

Disclosures: C. Bonthron: None. **M.J. Broadhead:** None. **J. Waddington:** None. **M. Lopez:** None. **F. Zhu:** None. **S.G.N. Grant:** None. **C. Smith:** None. **G. Miles:** None.

Poster

P291. Motor Neuron Disease: Animal Models I

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Program #/Poster #: P291.04

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant 5T32AG000255-24
NIH Grant 5R01NS110688-03

Title: When ALS processes are halted. motor neurons that resistant to pathological TDP-43 can mediate motor recovery.

Authors: *S. HUR[1], M. DOMINIQUE[1], M. FARAG[1], J. Q. TROJANOWSKI[2,1], K. J. SPILLER[3], V. M. LEE[1,2];
[1]Ctr. for Neurodegenerative Dis. Res. (CNDR), [2]Dept Pathol & Lab. Med., Univ. of Pennsylvania Sch. of Med., Philadelphia, PA; [3]Janssen R&D, LLC, Spring house, PA

Abstract: In intermediate disease stages, surviving motor neurons (MNs) can partially compensate for the initial loss of the most vulnerable MNs in amyotrophic lateral sclerosis (ALS) patients, but studying the nature of this compensation has been difficult. However, using transgenic mice with inducible *Tar-DNA binding protein-43* (TDP-43)-triggered ALS disease, we can now 1) identify the MNs that the mediate motor recovery after the first wave of fast-fatigable (FF)-type motor units are lost, and 2) determine how these MNs change over the course of continued disease. We have previously shown that slow motor units are disease-resistant in rNLS8 mice[1] and can recover from ALS-like disease when the hTDP-43ΔNLS transgene is turned off. In the present study, we use a viral tracing strategy to show that the disease resistant MNs sprout to innervate vacated motor end-plates of adjacent muscle fibers. Moreover, we find that these newly reinnervated motor units are resistant to axonal dieback during a second epoch of human *Tar-DNA binding protein-43* (hTDP-43)-triggered disease in a fast-type skeletal muscle (tibialis anterior, TA). We demonstrate that when the TA muscle is re-innervated by FF fibers (after a nerve crush experiment) there is no change in the ALS-like disease progression, but when SK3 positive slower MNs reinnervate TA (via cross-reinnervation surgery), these connections are now disease resistant. After a TDP-43-triggered loss and subsequent reinnervation, the resilience of the new connections is remarkable considering that these motor units have had to greatly expand their motor field and are now connecting to the muscle fibers that were initially the most disease-vulnerable. These results are particularly exciting given recent advances in ALS clinical programs aimed at targeting genetic drivers of disease. Taken together, these early results in a mouse model of TDP-43 proteinopathy offer hope that in the future, if the disease progression could be halted in ALS patients, those patients would likely regain some degree of their lost motor function over time.

Disclosures: S. Hur: None. **M. Dominique:** None. **M. Farag:** None. **J.Q. Trojanowski:** None. **K.J. Spiller:** None. **V.M. Lee:** None.

Poster

P291. Motor Neuron Disease: Animal Models I

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Topic: C.06. Neuromuscular Diseases

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Delaware IDeA Network of Biomedical Research Excellence (INBRE) Pilot Award: NIH-NIGMS: 5P20GM103446

Title: Tdp-43 triple mutation mouse model of als

Authors: *M. DOPLER, K. COX, S. AREZOUMANDAN, M. GITCHO;
Delaware State Univ., Dover, DE

Abstract: Amyotrophic Lateral Sclerosis (ALS) is characterized by the death of the upper and lower motor neurons. Symptoms include slurring of speech, difficult swallowing, progressive muscle weakness, and eventually paralysis. After diagnosis, the average lifespan is ~5 years, most commonly due to respiratory failure or pneumonia. There is currently no treatments for the disease. Over 95% of all ALS cases present hyper-ubiquitinated and phosphorylated transactive response DNA binding protein 43 (TDP-43) positive aggregates as part of the hallmark pathology. Replicating these hallmarks in a mammalian model is crucial in order to develop effective treatments. We have developed a new model of TDP-43 overexpression that contain three familial mutations (3XTDP43). When overexpressed in HEK293T and SHSY-5Y cells there are distinct changes in solubility producing phosphorylated TDP-43 aggregation. This leads us to developed a mouse that selectively expresses 3XTDP43 in the brain and spinal cord utilizing the neurofilament heavy promoter expressing tetracycline-controlled transactivator protein (NEFH-tTA). This mouse model demonstrates significant age-dependent motor deficits and mice only survive about 2 months of age. In addition, there are increases in neuroinflammation and phosphorylated TDP-43 pathology reminiscent of the human disease. This new mouse model may provide a better understanding of pathways associated ALS and other TDP-43 proteinopathies.

Disclosures: M. Dopler: None. **K. Cox:** None. **S. Arezoumandan:** None. **M. Gitcho:** None.

Poster

P291. Motor Neuron Disease: Animal Models I

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Program #/Poster #: P291.06

Topic: C.06. Neuromuscular Diseases

Support: NIH Grant NS091299
NIH Grant T34 GM08718

Title: Tdp-43 proteinopathy causes global dysregulation of neuronal protein synthesis in-vivo

Authors: *N. PENA, D. ZARNESCU, S. MACKLIN, C. CHELLMAN;
Univ. of Arizona, Tucson, AZ

Abstract: TAR DNA-binding protein 43 (TDP-43) is a DNA/RNA binding protein whose cytoplasmic aggregation is a pathological hallmark of amyotrophic lateral sclerosis (ALS). How disease-associated TDP-43 aggregates may exert toxicity within motor neurons, which rapidly deteriorate in ALS patients, is not fully understood. Given the important role TDP-43 plays in RNA metabolism and local translation within nerve cells, we investigated alterations in protein synthesis in *Drosophila* models of ALS based on wild-type or mutant TDP-43 overexpression in motor neurons. We found that global translation in neuronal cell bodies was reduced in the ALS fly models compared to controls, as indicated by puromycin labeling of nascent peptides. Additionally, genetic enhancement of translation initiation via 4EBP RNAi or S6K OE can rescue locomotor phenotypes induced by TDP-43 proteinopathy. Consistent with these results, wild-type animals show increased vulnerability to pharmacological inhibition of cap-dependent translation, which suggests a preexisting translational impairment in the *Drosophila* ALS models. When using fluorescent noncanonical amino acid tagging (FUNCAT) to label peptides in motor neurons, we also observe a reduction in newly synthesized proteins at the neuromuscular junction (NMJ) in flies overexpressing TDP-43. Finally, preliminary data suggest that both 4EBP RNAi and S6K OE can increase adult lifespan in the context of TDP-43 proteinopathy. Taken together, our data point toward compartment specific, cap-dependent protein synthesis impairment as a key process disrupted by TDP-43 pathology in-vivo.

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Poster

P291. Motor Neuron Disease: Animal Models I

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Program #/Poster #: P291.07

Topic: C.06. Neuromuscular Diseases

Support: ALS Ride for Life
NIA R01 1RF1AG057338

Title: Tdp-43 proteinopathy causes differential expression of retrotransposons in a tdp43- q331k mouse model

Authors: *S. KORADA[1], O. TAM[2], M. HAMMELL[2], D. WILLBOLD[3], J. DUBNAU[4], R. SHER[1];
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Spring Harbor, NY; [3]Heinrich Heine Univ., Dusseldorf, Germany; [4]Stony Brook Sch. of Med., Stonybrook University, NY

Abstract: Pathological cytoplasmic aggregation of TAR-DNA binding protein (TDP-43) is seen in 97% of ALS cases, 40% of FTD cases and many AD patients. TDP-43 is an RNA and DNA binding protein that has two RNA recognition motifs and an inherently disordered C- terminal domain that makes the protein prone to aggregation. TDP-43 is involved in many aspects of RNA metabolism such as transcriptional repression, translational regulation and mRNA splicing. TDP-43 has also been shown to regulate the expression of retrotransposable elements (RTEs). TDP-43 dysfunction, due to either over-expression, knock out, or the spontaneous proteinopathy seen in patients, result in elevated expression of RTEs. Studies in Drosophila in particular have demonstrated that the degenerative effects of TDP-43 are mediated by RTE toxicity to neurons and glia. The RTE phenomenology observed in Drosophila has also been observed in human subjects. Post- mortem RNA sequencing from ALS subjects, and analysis of chromatin silencing marks from FTD have revealed increased expression of human RTEs and endogenous retroviruses (ERVs), including HERV-K and LINE1. These changes in RTE expression are correlated with the degree of TDP-43 proteinopathy. While this phenomenology of RTE/ERV expression has been widely observed, and shown to provide a causal impact in Drosophila, there are as yet no mammalian model studies of the link between TDP-43 pathology, RTE expression, and toxicity. In this study, we tested the hypothesis that the TDP43-pathology impacts RTE expression in rodents. We used a mouse model with moderate levels of expression of the TDP-43 Q331K. We employed RNA-Seq analysis of total RNA from the motor cortex of transgenic mice overexpressing human TDP-43 Q331K, WT TDP-43, and their non- transgenic littermates. We found that expression of the Q331K transgene leads to derepression of several families of RTEs and ERVs. This effect was not observed in the animals expressing the TDP-43 WT protein. To correlate these effects with TDP-43 proteinopathy, we used surface-based fluorescence intensity distribution analysis (sFIDA) to detect TDP-43 oligomers at the pre-fibril stage in different regions of the brain and spinal cord. The TDP43 Q331K mutant mice show significantly higher levels of TDP-43 oligomers in the motor cortex. We conclude that there is an age- specific differential expression of retrotransposable elements in the motor cortex of the TDP-43 Q331K mouse model, which correlates with the degree of proteinopathy. This study provides a platform to investigate the mechanisms by which RTEs may contribute to neurodegeneration in this mammalian model of TDP- 43 proteinopathy.

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Poster

P291. Motor Neuron Disease: Animal Models I

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Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P291.08

Topic: C.06. Neuromuscular Diseases

Title: Characterization of TDP-43ΔNLS mice: Behavior and Biomarker Analyses

Authors: H. FERNANDES, I. MORGANSTERN, J. BELTRAN, M. KWAN, J. SHEA, A. GHAVAMI, D. HAVAS, *T. HANANIA;
Psychogenics Inc, Paramus, NJ

Abstract: Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease characterized by progressive loss of both upper and lower motor neurons and associated function, producing muscle denervation, motor impairments and brain atrophy leading to eventual death. One feature thought to correlate with ALS-related pathology is the deposition of insoluble cytosolic inclusions of TAR-DNA binding protein (TDP-43) in affected tissues. To study the progression of ALS-related phenotypes and establish a model for testing novel therapeutic interventions, we systematically characterized TDP-43ΔNLS mice (Walker et al., 2015). These inducible rNLS8 mice were generated by crossing a line of transgenic mice expressing tTA under the control of the human neurofilament heavy chain (NEFH) promoter with tetO-hTDP-43ΔNLS mouse containing a defective nuclear localization motif (DNLS). Administration of doxycycline (Dox) in these animals suppresses expression of both total and phosphorylated form of hTDP-43ΔNLS, and rescues the disease phenotype. Following Dox cessation, TDP-43ΔNLS mice show body weight loss, increased tremors, hindlimb clasping, impaired gait and decrease in survival compared to tTA control mice. Electromyography assessment showed an increase in the latency of muscular contraction and a decrease in the response amplitudes of muscle contractions following motor nerve stimulation in TDP-43ΔNLS animals. Histological analysis revealed strong overexpression of TDP43 in perinuclear cytoplasmic inclusions along with deposition of pTDP43 aggregates in hippocampus, cerebral cortex, dorsal striatum and cerebellum of TDP-43ΔNLS mice. TDP pathologies were accompanied by increased expression of inflammatory marker transcripts in cortex, astrogliosis and microglial activation in affected brain regions of TDP-43ΔNLS mice compared to tTA controls. Similar type of pathologies were detected in the spinal cord but at lower level of severity than in the brain. Dramatic elevations in neurofilament light chain, a biomarker of neurodegeneration, were seen in both plasma and CSF of TDP-43ΔNLS animals 5 weeks following withdrawal of Dox. In summary, expression of hTDP-43ΔNLS in rNLS8 mice resulted in cytoplasmic inclusions in multiple brain regions and spinal cord, a loss of murine nuclear TDP-43, progressive loss of muscle strength and function, and motor impairments. Each of these impairments were observed early after Dox removal in both male and female mice suggesting that this mouse model of ALS provides an aggressive and clinically-relevant phenotype for testing novel therapeutic strategies.

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Poster

P291. Motor Neuron Disease: Animal Models I

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Program #/Poster #: P291.09

Topic: C.06. Neuromuscular Diseases

Title: Neuronal cytoplasmic TDP-43 accumulation leads to spinal cord lipid cacostasis in the rNLS8 mouse model of amyotrophic lateral sclerosis

Authors: *E. JENSEN[1], E. MCGUIRK[4], L. GUO[2], T. TAKSIR[3], Y. REN[1], A. BIALAS[1], J. YU[1], S. RYAN[3], D. OFENGEIM[1], B. ZHANG[1], P. SARDI[1], J. DODGE[1];
[1]Rare and Neurolog. Dis. Therapeut. Area, [2]Translational Sci., [3]Translational In Vivo Models, Sanofi Genzyme, Framingham, MA; [4]Dept. of Biol., Brandeis Univ., Waltham, MA

Abstract: TAR DNA-binding protein 43 kDa (TDP-43) is a highly conserved and ubiquitously expressed predominantly nuclear RNA-binding protein that performs important functions in transcription, translation, pre-mRNA splicing, and RNA transport to impact the expression of thousands of genes. Intracellular aggregation and accumulation of TDP-43 is a neuropathological hallmark observed in 97% of patients with amyotrophic lateral sclerosis (ALS) and 50% of patients with frontotemporal dementia (FTD). These neurodegenerative diseases are devastating for patients and are a growing societal burden. Transformative therapies are currently unavailable to patients living with ALS and FTD; thus, a deeper more complete understanding of the underlying mechanisms that trigger TDP-43 dependent neurodegeneration is urgently needed. Previously, we showed that lipid cacostasis occurs in the spinal cords of ALS patients and $SOD1^{G93A}$ mice, a familial mouse model of ALS. Here, we determined if TDP-43 mediated neurodegeneration in rNLS8 mice, a mouse model of motor neuron disease, is also associated with spinal cord lipid cacostasis. We used LC-MS to investigate the lumbar spinal cords of rNLS8 mice for potential subcellular (cytoplasmic vs. nuclear) changes in lipids. Consistent with previous studies, we demonstrate that induction of cytoplasmic TDP-43 accumulation at 3 months of age resulted in significant motor impairment in nestlet building, rotarod, and wirehang starting at 1 week. The rNLS8 mice displayed rapid disease progression with a significant loss of total, fat, and muscle mass at 3 weeks and a median time to death of 7.6 weeks. Starting at 16 days after disease induction, we measured increased levels of the full-length protein, C-terminal fragments, and S409/410 phosphorylated isoforms of TDP-43 in the cytoplasmic fraction of the lumbar spinal cord and increases in neurofilament-heavy in the serum suggesting neuronal loss. Finally, using lipidomics, we observed significant subcellular dysregulation of lipids in the lumbar spinal cord. Altogether, these results confirm that cytoplasmic TDP-43 accumulation leads to rapid progressive motor neuron disease and implicate nuclear/cytoplasmic lipid cacostasis as an important mechanism in disease progression.

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part-time):; Sanofi. **P. Sardi:** A. Employment/Salary (full or part-time):; Sanofi. **J. Dodge:** A. Employment/Salary (full or part-time):; Sanofi.

Poster

P291. Motor Neuron Disease: Animal Models I

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Program #/Poster #: P291.10

Topic: C.06. Neuromuscular Diseases

Support: NIH R21 DC016071
NIH 1R01HL153612-01

Title: Impact of limb phenotype on tongue denervation atrophy, dysphagia penetrance, and survival time in a mouse model of ALS

Authors: *R. THOMPSON[1], M. MUELLER[2], K. OSMAN[2], S. KINGTON[2], Z. STEPHENSON[2], A. HAMAD[2], F. BUNYAK[2], N. L. NICHOLS[3], T. E. LEVER[4];
[1]Univ. of Missouri, Columbia, Columbia, MO; [3]Dept. of Biomed. Sci., [2]Univ. of Missouri, Columbia, MO; [4]Otolaryngology - Head and Neck Surgery, Univ. of Missouri Columbia, Columbia, MO

Abstract: Introduction
Dysphagia in ALS is predominantly attributed to progressive tongue weakness and atrophy, ultimately rendering patients unable to eat/drink by mouth. Current treatments are symptom-based (e.g., diet modification, feeding tube) and do not target the underlying tongue denervation atrophy. To address this clinical need, we are studying the low copy number SOD1-G93A (LCN-SOD1) mouse model of ALS with dysphagia and limb phenotype variability resembling human ALS. Our main goal was to quantify the impact of limb phenotype on tongue denervation atrophy, dysphagia penetrance, and survival time in preparation for future treatment-based studies. Methods Two male LCN-SOD1 breeders and 125 transgenic and wild-type (WT) offspring underwent behavioral testing for heritability of limb phenotype. Fifty-two offspring (30 LCN-SOD1 and 22 WT, both sexes) were randomly selected for characterization of dysphagia penetrance (via videofluoroscopic swallow study; VFSS) and survival time at disease end-stage (15-20% body weight loss). From these, 16 mice (8 LCN-SOD1 and 8 WT) underwent postmortem histological analysis of the tongue (genioglossus) for microscopic evidence of denervation atrophy. Results Both male breeders displayed a mixed ALS phenotype (i.e., hindlimb *and* forelimb involvement) at disease end-stage and sired equal proportions of mixed vs. hindlimb phenotype offspring. Both LCN-SOD1 phenotypes had significant dysphagia compared to WT mice ($p < 0.05$): slower lick and swallow rates, longer inter-lick and inter-swallow intervals, and longer pharyngeal transit times. Dysphagia penetrance differed between phenotypes (mixed = 100%; hindlimb = 64%), yet survival times were similar. LCN-SOD1

mice, regardless of phenotype, had significantly smaller genioglossus myofibers and more centralized myonuclei compared to WT mice ($p < 0.05$). These biomarkers of denervation atrophy were significantly correlated with VFSS metrics (lick and swallow rates, $p < 0.05$) but not survival time. Conclusions Limb phenotype heritance was only chance level for LCN-SOD1 mice, whereas dysphagia penetrance was complete for mixed (100%) versus incomplete for hindlimb (64%) phenotype mice. Remarkably, both phenotypes had tongue denervation atrophy, even hindlimb phenotype mice without dysphagia. Unlike human ALS, LCN-SOD1 mice with dysphagia and tongue atrophy did not have shorter survival times, likely due to our humane end-point criterion. Nonetheless, this mouse model recapitulates key aspects of human ALS and therefore provides a translational platform to accelerate the discovery of targeted treatments for tongue denervation atrophy and dysphagia.

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Poster

P305. Neuroinflammation: Neurodegeneration III

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Program #/Poster #: P305.01

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

Support: NSF-GRFPDGE-1644869
1 R25 AG059557-01

Title: Investigating caspase expression and cellular localization in human retinal vascular disease

Authors: *L. RUSS[1], C. K. COLON ORTIZ[2], A. M. POTENSKI[2], C. M. TROY[3];
[1]North Carolina State Univ., Raleigh, NC; [2]Columbia Univ., New York City, NY; [3]Dept Pathol & Neurol, Columbia Univ. Medl Ctr., New York, NY

Abstract: Neurovascular injury, characterized by the breakdown of the blood-retina barrier and inflammation, has been associated with neurodegeneration. One treatment of retinal neuroinflammation targets vascular endothelial growth factor (VEGF), an inflammatory cytokine that promotes neovascularization and angiogenesis. While anti-VEGF treatments prevent neovascularization and edema, they do not prevent neuronal loss and subsequent decline in vision. Caspases, proteases that are associated with cell death pathways, increase in CNS tissues because of hypoxia-ischemia influencing neuronal death, a hallmark of neurodegeneration. Studies in a mouse model of vascular disease have indicated that activation of caspase-9 induces retinal neuronal death, and its inhibition reduces inflammation and neuronal damage. This

project's objective is to evaluate how caspase-9 expression and cellular localization in anti-VEGF treated and untreated post-mortem human retinas compares to a mouse model of neurovascular injury, retinal vein occlusion. The neuroretina is part of the CNS therefore allowing retinal findings to translate to neurological disease of the brain. The analysis tested the hypothesis that caspase-9 and caspase-7 signaling in anti-VEGF treated post-mortem human retinas will mirror that of uninjured WT mice. We collected four anti-VEGF treated and non-treated human retinas with different vascular diseases, as well as control retinas without vascular disease and immunostained them for caspases-9 and -7 and for cellular markers for endothelial cells and glial cells. Confocal images were obtained and processed via ImageJ. Our results emphasize the importance of evaluating the translational significance of anti-VEGF treatments when developing potential therapeutics for retinal vascular diseases.

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Poster

P305. Neuroinflammation: Neurodegeneration III

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Program #/Poster #: P305.02

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

Support: NIH/NIDCD Grant R01 DC015790

Title: Cochlear neurodegeneration in deafened rats involves an immune response not requiring complement

Authors: *B. M. GANSEMER, S. H. GREEN;
Dept. of Biol., Univ. of Iowa, Iowa City, IA

Abstract: Background: Spiral ganglion neurons (SGNs) transmit information about acoustic stimuli from cochlear hair cells, the auditory receptor cells, to the CNS. SGNs slowly degenerate after hair cell loss but the reason for this degeneration is unclear because neurotrophic support remains available. Our RNA-seq and histological studies show an immune response, including complement activation, in the spiral ganglion after hair cell loss. We assessed whether complement activation contributes to SGN death using a rat model in which complement component 3 (C3) was knocked out. We assessed SGN survival in hearing and deafened $C3^{+/+}$ and $C3^{-/-}$ rats. Additionally, we analyzed histologically infiltration and activation of immune cells in the spiral ganglion.
Methods: Male and female Sprague-Dawley rats were intraperitoneally injected with kanamycin once daily from postnatal day 8 (P8) through P16 to destroy hair cells. Cochleae were collected and spiral ganglia isolated at P32 or P60 for RNAseq (6-8 ganglia per replicate). The RNAseq

data were analyzed using available computational tools and software packages. For histological analyses, cochleae from hearing and deafened $C3^{+/+}$, $C3^{+/-}$, and $C3^{-/-}$ rats were collected at P70 and cryosectioned parallel to the midmodiolar plane at 25 μm thick. Sections were labeled with antibodies to identify neurons (Tuj1), macrophages (IBA1), activated macrophages (anti-CD68), or CD4 immunoreactive cells.
Results: Gene set enrichment analysis (GSEA) revealed that the largest functional category enriched after deafening contained genes associated with an immune response. More specifically, genes involved in the complement cascade, chemotaxis, and antigen presentation were upregulated after deafening. Histological analysis revealed infiltration of macrophages and increased expression of CD68, a marker of phagocytic activity and cell activation, in the deafened ganglia. Moreover, the immune response includes not only monocytes but also CD4+ lymphocytes. However, C3 knockout did not affect SGN survival, indicating that complement activation does not play a role in SGN death after deafening.
Conclusions: Many immune response genes, including complement system genes, are upregulated in the spiral ganglion after hair cell loss, consistent with infiltration and activation of monocytes and lymphocytes. However, SGN death and IBA1+ cell infiltration were similar in $C3^{+/+}$ and $C3^{-/-}$ rats, implying that complement activation does not play a role in SGN death. The data do suggest that adaptive, as well as innate, immune responses are involved.

Disclosures: B.M. Gansemer: None. **S.H. Green:** None.

Poster

P305. Neuroinflammation: Neurodegeneration III

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P305.03

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

Support: AHA 15BGIA2509029
VA CDA KBX003240
San Antonio Nathan Shock Center Pilot Grant

Title: Lrp1 as a modulator of neurotoxic astrocyte activation after stroke and traumatic brain injury

Authors: S. N. AHMAD[1], ***N. L. SAYRE**[2];
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Abstract: Changes in gene expression patterns of glial cells can predict brain age[1] suggesting that glial function is a major contributor to changes in cognition during aging. Moreover, a history of brain damage such as from ischemic stroke or traumatic brain injury (TBI) increase the

likelihood of developing dementia or cognitive decline. **Our goal is understand a potential mechanism which underlies age-associated changes in astrocyte physiology.** With aging, astrocytes undergo a series of gene expression changes, switching their neurotrophic role to a neurotoxic role[2]. Neurotoxic reactive astrocytes have disrupted ability to promote synaptic function and improve neuronal survival after stress[3] and are induced by microglial secretion of Interleukin-1α (IL1α), Tumor necrosis factor (TNF), and Complement component C1q[3]. We have discovered a potential astrocyte mediator, Low-density lipoprotein receptor related protein 1 (**LRP1**). Located on the plasma membrane, LRP1 uses receptor mediated endocytosis to escort proteins to the endocytic system for degradation, associates with signaling complexes, and is cleaved to regulate nuclear signaling[4]. We discovered that loss of LRP1 causes increased astrocyte sensitivity to TNFα. Others found that LRP1 regulates components of the complement system,[5] and interferes with IL1 receptor signaling[6], altogether suggesting LRP1 could directly affect astrocyte sensitivity to microglia signals that activate neurotoxic astrocytes. We used an inducible Connexin-30 Cre to knockout LRP1 in astrocytes of adult mice. Mice were subjected to middle cerebral artery occlusion (MCAO) or repetitive traumatic brain injury and were aged for up to a year after injury. We discovered astrocyte KO of LRP1 had *improved* long-term behavioral outcomes after MCAO yet were *worsened* after repetitive TBI. We also found decreased astrocyte gliosis after MCAO. We think that these differential outcomes could be due to differential neurotoxic astrocyte activation. To test this, we are performing protein and gene expression studies in astrocytes lacking LRP1, while simultaneously interrogating the effect of LRP1 on neurotoxic astrocyte activation *in vivo* and in aged mouse models.
These studies were funded by: AHA 15BGIA2509029, VA CDA KBX003240, and a San Antonio Nathan Shock Center Pilot Grant (N.L.S).
1. Soreq, L. *et al. Cell reports* **18**, 557-570. (2017).
2. Clarke, L. E. *et al. PNAS* **115**, E1896-E1905 (2018).
3. Liddelow, S. A. *et al. Nature* **541**, 481-487. (2017).
4. Mao, H., Xie, L. & Pi, X. *Front Cardiovasc Med* **4**, 34-34. (2017). 5. Gaultier, A. *et al. Blood* **111**, 5316-5325. (2008).
6. Kawamura, A. *et al. Arterioscler Thromb Vasc Biol* **27**, 1610-1617. (2007).

Disclosures: S.N. Ahmad: None. **N.L. Sayre:** None.

Poster

P305. Neuroinflammation: Neurodegeneration III

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Program #/Poster #: P305.04

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

Support: NIH Grant R01NS112291
MDA

Title: Transcriptome Alterations in Myotonic Dystrophy Frontal Cortex

Authors: *B. OTERO[1], K. POUKALOV[2], R. P. HILDEBRANDT[2], E. T. WANG[2];
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Abstract: Myotonic dystrophy (dystrophia myotonica, DM) is caused by expanded CTG/CCTG microsatellite repeats, leading to multi-systemic symptoms in skeletal muscle, heart, gastrointestinal, endocrine, and central nervous systems (CNS), among others. For some patients, CNS issues can be as debilitating or more so than muscle symptoms; they include hypersomnolence, executive dysfunction, white matter atrophy, and neurofibrillary tangles. Although transcriptomes from DM type 1 (DM1) skeletal muscle have provided useful insights into pathomechanisms and biomarkers, limited studies of transcriptomes have been performed in the CNS. To elucidate underlying causes of CNS dysfunction in patients, we have generated and analyzed RNA-seq transcriptomes from the frontal cortex of 21 DM1 patients, 4 DM type 2 (DM2) patients, and 8 unaffected controls. One hundred and thirty high confidence splicing changes were identified, most occurring exclusively in the CNS and not in skeletal muscle or heart. Mis-spliced exons were found in neurotransmitter receptors, ion channels, and synaptic scaffolds, and we identified an alternative exon in GRIP1 that modulates association with kinesins. Splicing changes exhibited a gradient of severity correlating with CTG repeat length, as measured by optical mapping of individual DNA molecules. All individuals studied, including those with modest splicing defects, showed extreme somatic mosaicism, with a subset of alleles having >1000 CTGs. Analyses of gene expression changes showed up-regulation of genes transcribed in microglia and endothelial cells, suggesting neuroinflammation, and down-regulation of genes transcribed in neurons. Gene expression of RNAs encoding proteins detectable in cerebrospinal fluid were also found to correlate with mis-splicing, with implications for CNS biomarkers of disease severity. These findings provide a framework for future mechanistic and therapeutic studies of CNS issues in DM.

Disclosures: B. Otero: None. **K. Poukalov:** None. **R.P. Hildebrandt:** None. **E.T. Wang:** None.

Poster

P305. Neuroinflammation: Neurodegeneration III

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Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P305.05

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

Support: IND2018/BMD-9751

Title: Remyelinating Multiple Sclerosis: effects of TLR4 blocking aptamer ApTOLL in murine EAE

Authors: *B. FERNANDEZ GOMEZ[1], D. PIÑEIRO[2], M. HERNÁNDEZ[2], P. GÓMEZ-MARTÍN[1], F. DE CASTRO[1];
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Abstract: Multiple sclerosis (MS) is a degenerative and autoimmune chronic disease of the central nervous system (CNS) that constitutes the second cause of neurological disability in young adults. MS is characterized by the death of oligodendrocytes and the subsequent loss of myelin in both, the white and the gray matters. The autoimmune component of MS causes inflammation, demyelination, and axon. The activation of Toll-like type 4 receptor (TLR4) acts as a potent proinflammatory signal. ApTOLL is an innovative molecule developed with aptamer technology. ApTOLL is known to antagonize TLR4, generating an anti-inflammatory effect in stroke. This single chain DNA aptamer represents a novel strategy for its nature and mechanism of action to treat diseases with an important inflammatory component, such as MS. Our current research shows the immunomodulatory plus remyelinating effect of four doses of ApTOLL in the Experimental autoimmune encephalomyelitis (EAE) model of MS. A single-dose treatment at the onset of symptoms significantly ameliorates the clinical score, as well as significantly positive effects were observed in myelin and neurofilaments *in vivo*. Furthermore, this molecule seems to have a direct effect on the biology of oligodendrocytes precursors cells (OPCs) by promoting their proliferation and differentiation towards myelinating phenotypes *in vitro*. ApTOLL by itself and/or this effect combined with other neuroprotectors could be a highly innovative strategy that would cover all aspects of the ideal therapy for MS.

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Poster

P305. Neuroinflammation: Neurodegeneration III

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Program #/Poster #: P305.06

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

Support: NIH/NIA R01AG060195
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Title: Neuroinflammatory responses to viral-like stimuli causes alpha-synuclein aggregation and tau hyperphosphorylation in vivo with long-term changes to synaptic and vesicular transport proteins.

Authors: *O. BREKK, R. THOMAS, **K. J. CONNOLLY**, C. CLOUSE, S. ROSENTHAL, Z. K. MACBAIN, A. PERUGINI, C. M. DOUALA, P. J. HALLETT, O. ISACSON;
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Abstract: In animal models, pro-inflammatory drivers mimicking either bacterial (lipopolysaccharide; LPS) (Koprich et al., 2008) or viral (polyinosinic:polycytidylic acid; POLY(I:C)) (Deleidi et al., 2010) have been shown to sensitize dopaminergic neurons to subsequent noxious stimuli in "double-hit" models of Parkinson's Disease (PD) pathogenesis. These observations indicate that host defense mechanisms -- if not physiologically resolved, and potentially in combination with genetic or environmental risk factors -- can precipitate neurodegenerative events leading to disease. To investigate early brain changes associated with acute anti-viral inflammatory responses, we unilaterally delivered the synthetic dsRNA toll-like receptor 3 (TLR3) agonist POLY(I:C) to the hippocampus and substantia nigra of WT mice. We assessed pre-degenerative changes by histology and biochemical assays at days 2, 12, and 33 post-injection, with daily behavioral readouts of sickness behavior. The results demonstrated increased proteolytic cleavage of C3 with accumulation of C1q protein levels at the 2-day time-point, with alpha-synuclein aggregation and concurrent Tau hyperphosphorylation in the ventral midbrain and hippocampus of Poly(I:C)-injected mice. There were signs of increased ApoE levels at 2 days, but not at 12 and 33 days. From day 16 onwards, the animals exhibited increased sickness behaviors which persisted until the study endpoint. Markers of axonal integrity and synaptic proteins were also studied, and the results will be reported. Additionally, to study prolonged states of chronic inflammation, WT mice were injected intra-peritoneally with POLY(I:C) for 12 consecutive months (with the last Poly(I:C) injection being at 11 months). Preliminary data suggest there is a degenerative change of the dopaminergic neurons of the SNpc at the 12-month time-point with changes related to axonal integrity and anterograde synaptic transport, and some increase of synaptic structural proteins. These attempts to mirror viral infection-induced neuronal and synaptic changes, indicate that factors associated with degeneration in PD and related brain degenerative disorders, are found in the substantia nigra and hippocampus with intra-parenchymal and systemic TLR3 stimulation.

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Poster

P305. Neuroinflammation: Neurodegeneration III

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

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Title: Cerebral blood flow is reduced and brain R_2* is increased in the lipopolysaccharide (LPS) model of inflammation: An in-vivo 9.4T MRI study

Authors: *Q. SHAFQAT[1,2,3], Y. WU[1,2,3], A. HAMILTON[1,2,3], M. HASHEM[1,2,3], J. F. DUNN[1,2,3];
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Abstract: Inflammation is associated with a wide range of neurological disorders. It is likely that systemic inflammation can induce inflammation in the central nervous system. Reductions in cerebral blood flow (CBF) have been reported in animal models of inflammation and in multiple sclerosis, which has an inflammatory component. Reductions in CBF have also been linked to hypoxia (low oxygenation) and declines in cognitive and behavioral function. Therefore, it is important to study the link between CBF, hypoxia, behavior, and inflammation. Here, we investigated the relationship between CBF, relaxation rate (R_2*) which is a qualitative marker of deoxyhemoglobin (dHb), and behavior in a model of systemic inflammation induced by bacterial lipopolysaccharide (LPS).

Female C57BL/6 mice received 2 mg/kg LPS (n=11) or saline (n=10) via i.p. injection once a day for 3 consecutive days. Behavior was assessed at baseline (pre-treatment) and once daily post-treatment using the open-field (OF) test. Mice were imaged in-vivo with 9.4T MRI 3 hours post-treatment on Day 3 to measure CBF and R_2*. CBF and R_2* were quantified in 7 regions. A Student's *t*-test was used to compare between control and LPS treated groups with an FDR-correction (Benjamini-Hochberg) for multiple comparisons. Linear relationships were assessed with Pearson correlation.

Compared to controls, LPS treated mice experienced significant reductions in CBF in the cortex ($p<0.001$), right and left hippocampus ($p<0.03$), right piriform cortex ($p<0.01$), and left piriform cortex ($p<0.001$). LPS also resulted in increases in R_2* in the cortex ($p<0.05$), right and left hippocampus ($p<0.05$), and right piriform cortex ($p<0.01$). R_2* increase is consistent with increase in tissue deoxyhemoglobin (dHb). Cortical CBF and R_2* were negatively correlated ($p<0.05$, $R= -0.54$). Locomotion declined significantly in the LPS group by Day 2 and 3 post-treatment ($p<0.001$). Locomotion on Day 2 positively correlated with CBF in the cortex and right piriform cortex ($p<0.01$, $R= 0.61$; $p<0.05$, $R= 0.49$, respectively), and negatively correlated with

R_2* in the cortex ($p<0.05$, $R= -0.541$).
We report regional reductions in CBF alongside increases in R_2*, which is consistent with elevated dHb. Cerebral hypoperfusion coupled with elevated dHb could be associated with abnormal oxygen delivery or increased oxygen extraction by inflamed tissues. Reductions in CBF and increases in brain R_2* also correlated with a decline in locomotion. These data support the concept that inflammation can cause hypoxia in the brain as well as a reduction in blood flow, which relate to behavioral impairment.

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Poster

P305. Neuroinflammation: Neurodegeneration III

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Program #/Poster #: P305.08

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

Support: NIH Grant NS101167
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CURE Epilepsy

Title: Pharmacological inhibition of the inflammatory receptor CCR2 relieves the immediate deleterious consequences of status epilepticus

Authors: *N. VARVEL, C. ALEMAN-RUIZ, W. WANG, R. J. DINGLEDINE;
Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Generalized status epilepticus (SE) triggers a robust neuroinflammatory response involving reactive astrocytosis, activation of brain-resident microglia, and brain infiltration of CCR2+ monocytes. Multiple lines of evidence indicate that quenching SE-induced neuroinflammation can alleviate the adverse consequences of SE, including neuronal damage and cognitive impairments. Our recent findings show that blocking monocyte brain entry after SE, via global *Ccr2* KO, rescues several SE-induced adverse effects including blood-brain barrier (BBB) erosion and neuronal damage while enhancing weight regain. The goals of the present study were to determine if CCR2 antagonism with a small molecule after SE blocks monocyte brain entry, dampens brain inflammation, and provides neuroprotection three days after SE. Eight- to ten-week-old male *Ccr2*$^{+/rfp}$ heterozygous mice maintained on the C57BL/6NCrl inbred genetic background were subject to intraperitoneal injection of kainic acid (KA), scored for seizure severity, weight recovery, nest building capability, and sacrificed three days post-SE. Surviving mice were randomized into CCR2 antagonist (n=19) and vehicle (n=20)

groups. The CCR2 antagonist, or vehicle, was administered 24- and 48- hours post-SE via oral gavage. Mice subject to the CCR2 antagonist displayed faster weight recovery between one- and three-days post-SE and enhanced ability to build a nest on the third day after SE when compared to their vehicle-treated controls. The mRNA levels of a panel of inflammatory mediators was depressed by a mean of 47%, and the glial markers were reduced by 30% in mice treated with the CCR2 antagonist compared to controls. CCR2 antagonism provided neuroprotection and astrocytosis was reduced in four brain regions, but the area covered by the macrophage marker, Iba1, was not significantly reduced by the CCR2 antagonist. Our findings further confirm that inflammatory processes are pathogenic after SE, provide proof-of-concept that CCR2 antagonism after SE can alleviate multiple adverse effects, and identify CCR2+ monocytes as a viable therapeutic target.

Disclosures: N. Varvel: None. **C. Aleman-Ruiz:** None. **W. Wang:** None. **R.J. Dingledine:** None.

Poster

P310. Neuroprotective Mechanisms: Preclinical: Other Therapeutics II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P310.01

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

Title: Neuroprotective effects of Maqui Berry on blood-brain barrier damage in a model of Pentylenetetrazole-induced recurrent seizures in old rats.

Authors: *O. GARCÍA[1,2], A. VEGA[3], C. GARCÍA[4], D. A. MORENO[4], S. A. OROZCO[1]; [1]Unit of Med. Res. in Neurolog. Dis., Mexican Inst. of Social Security, Mexico, Mexico; [2]Dept. of Biochem., Natl. Polytechnic Inst., Mexico city, Mexico; [3]Dept. of Physiol., Natl. Autonomous Univ. of Mexico, Mexico City, Mexico, Mexico; [4]Dept. of Food Sci. and Technol., Univ. Campus of Espinardo Building 25, Murcia, Spain, Spain

Abstract: Rationale: The aging process progressively deteriorates health and quality of life, increasing susceptibility to seizures. Additionally, blood-brain barrier (BBB) dysfunction has been reported as a cause of the development of seizure activity. The Maqui Berry (MB), *Aristotelia chilensis*, contains flavonoids with strong antioxidant activity; the inhibition of oxidative stress has anticonvulsant effects. **Goal:** To assess the neuroprotective effect of MB administration on BBB damage and seizure susceptibility in old rats. **Methods:** Male *Sprague dawley* rats, 12 months of age, were administered 100 mg/kg of MB, orally, for 20 days before and during the evaluation of seizure susceptibility; a subthreshold dose of Pentylenetetrazol (PTZ) 55 mg/kg (i.p) was applied, volume of 1 ml/kg of body weight three times in 2-day intervals between each evaluation. The BBB proteins, iNOS, Cox-2 and Zo1, were evaluated by Western blot and immunofluorescence in cerebral capillaries, as well as the expression of gliosis

and neuronal survival in the ventral hippocampus using GFAP and Neun, respectively. **Results:** The MB group showed an increase in the latencies of generalized seizures, a higher expression of Zo1 proteins ($p < 0.001$), a lower expression of iNOS, Cox-2 in cerebral capillaries compared to the PTZ control group, a reduction in gliosis and greater neuronal survival in CA3 and GD. **Conclusion:** The antioxidant properties of MB protect the BBB and seizure susceptibility, which demonstrates its potential use as a neuroprotector in the adult brain.

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Poster

P310. Neuroprotective Mechanisms: Preclinical: Other Therapeutics II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P310.02

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

Support: 1R01NS106907

Title: Nsc-derived ev treatment in late middle age improves cognitive and mood function with antioxidant, antiinflammatory and neurogenic effects

Authors: *R. UPADHYA, S. ATTALURI, A. VOGEL, M. KODALI, M. LEELAVATHI NARAYANA, B. SHUAI, A. K SHETTY;
Inst. For Regen Med, Texas A&M Univ. Coll Med., College Station, TX

Abstract: Age-related cognitive dysfunction is typically associated with increased oxidative stress, moderate chronic neuroinflammation, and substantially decreased hippocampal neurogenesis. In the current study, we examined the effectiveness of intranasal (IN) administration of EVs generated from the rat subventricular zone neural stem cells (SVZ-NSCs) for improving cognitive and memory function in a mouse model of aging. The EVs from the SVZ-NSC culture media were first purified by anion-exchange chromatography and then characterized using NanoSight and markers of EVs. The anti-inflammatory property of EVs employed in this study was validated through an LPS-stimulated in vitro macrophage assay. A small RNA-*seq* study also confirmed that the EVs were enriched with miRs capable of mediating anti-inflammatory and neurogenic properties. Mice were treated intranasally with SVZ-NSC-EVs once weekly for three weeks (50 billion per administration) or vehicle starting from 18.5 months of age. A month later, the animals were interrogated with a series of behavioral tests to assess cognitive and mood function, following which the brain tissues were examined for oxidative stress, neuroinflammation, and neurogenesis. Vehicle-treated aged animals exhibited cognitive impairment for discerning a minor change in the environment in an object location test and distinguishing similar but not identical experiences in a non-overlapping fashion in a pattern

separation test. These animals also exhibited spatial memory dysfunction in a water maze test and anhedonia in a sucrose preference test. Remarkably, aged animals receiving NSC-EVs showed improved cognitive and mood function in all tests performed in the study. Furthermore, NSC-EV treatment normalized elevated concentrations of oxidative stress markers malondialdehyde and protein carbonyls and the proinflammatory cytokine IL-1 beta. NSC-EV treatment also stimulated increased production of the anti-inflammatory protein IL-10 and the antioxidant superoxide dismutase. Additional characterization demonstrated modulation of neuroinflammation typified by reduced astrocyte hypertrophy and activated microglia, and enhanced hippocampal neurogenesis in NSC-EV-treated animals. Thus, intranasal administration of NSC-EVs in late middle age improves cognitive and mood function, associated with the modulation of oxidative stress, neuroinflammation, and neurogenesis in the hippocampus.

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Poster

P310. Neuroprotective Mechanisms: Preclinical: Other Therapeutics II

Location: Virtual Only

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Program #/Poster #: P310.03

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

Title: Iduronate-2-sulfatase transport vehicle restores peripheral and central manifestations of MPS II disease in a mouse model of Hunter Syndrome

Authors: A. ARGUELLO, H. N. NGUYEN, E. R. THOMSEN, B. TSOGTBAATAR, M. FANG, S. S. DAVIS, I. A. LOPEZ, R. MEISNER, A. BHALLA, C. S. MAHON, M. S. KARIOLIS, A. G. HENRY, J. M. HARRIS, P. E. SANCHEZ, *K. SCEARCE-LEVIE; Denali Therapeut., South San Francisco, CA

Abstract: Mucopolysaccharidosis type II (MPS II) is a lysosomal storage disorder caused by deficiency of the iduronate-2-sulfatase (IDS) enzyme, resulting in cellular accumulation of glycosaminoglycans (GAGs) throughout the body. Treatment of MPS II remains a considerable challenge as current enzyme replacement therapies do not adequately control many aspects of the disease, including skeletal and neurological manifestations. We developed an IDS transport vehicle (ETV:IDS) that is engineered to bind to the transferrin receptor; this design facilitates receptor-mediated transcytosis of IDS across the blood-brain barrier and improves its distribution into the brain while maintaining distribution to peripheral tissues. We have leveraged the MPS II mouse model to discover clinically translatable biomarkers and assess target, pathway, and disease-relevant changes following ETV:IDS administration. Here we demonstrate that chronic systemic administration of ETV:IDS reduced levels of peripheral and central nervous system (CNS) GAGs, reduced CNS lysosomal lipids, and reduced microgliosis in the brain of MPS II

mice. Additionally, ETV:IDS rescued auricular and skeletal abnormalities when introduced in adult MPS II mice. These effects were accompanied by improvements in several neurobehavioral domains including motor skills, sensorimotor gating, and learning and memory. Using fluid-based biomarkers, we also observed increases in markers of neuroinflammation, neurodegeneration, and bone dysplasia in this mouse model, some of which were reduced following administration of ETV:IDS. Altogether, these results highlight the therapeutic potential of ETV:IDS for treating peripheral and central abnormalities in MPS II and supports the use of these clinically translatable biomarkers to monitor MPS II disease progression and treatment outcomes with therapies such as ETV:IDS. DNL310, an investigational ETV:IDS molecule, is currently in clinical trials as a potential treatment for patients with MPS II.

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Poster

P310. Neuroprotective Mechanisms: Preclinical: Other Therapeutics II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P310.04

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

Support: NS103350- 02S1
Xiangya Scholarship Fund

Title: Targeting regulatory T cells to treat nerve injury-induced dynamic mechanical allodynia

Authors: *R. HU[1], J. ZHANG[2], X. LIU[1], D. HUANG[3], Y. CAO[1];
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Abstract: Abstract Dynamic mechanical allodynia, the pain evoked by light brushing or stroking of the skin, is one of the most disabling symptoms of neuropathic pain. Despite the recent progress in elucidating the underlying mechanisms, effective treatment of dynamic allodynia remains elusive. Previous studies indicate that the immunosuppressive regulatory T (Treg) cells modulate the magnitude of punctate mechanical allodynia resulting from sciatic nerve injury. However, whether enhancing Treg-mediated suppression attenuates dynamic allodynia is not known. Daily low-dose interleukin-2 (ld-IL2) treatment has been used in many preclinical and clinical studies to preferentially expand and activate the endogenous Treg cells in peripheral tissues. In the present study, we investigated whether ld-IL2 treatment and Treg cell transfer are effective for the prolonged punctate and dynamic allodynia resulting from unilateral spared nerve injury (SNI) in mice. Daily ld-IL2 treatment from 5 days before SNI to 21 days post-injury completely blocked the development of SNI-induced dynamic mechanical allodynia and reduced the magnitude of punctate allodynia in female Swiss Webster mice. Delaying the initiation of ld-IL2 treatment to 7
days post-injury significantly attenuated the morphine-resistant punctate and dynamic allodynia at 3-5 weeks post-SNI. Furthermore, adoptive transfer of Treg cells to male C57BL/6J mice 3 weeks post-SNI effectively reversed the persistent punctate and dynamic allodynia, supporting that the effect of ld-IL2 is mediated through endogenous Treg cells, and is likely independent of mouse strain and sex. Neither ld-IL2 treatment nor Treg transfer affected the basal responses to punctate or brush stimuli. To elucidate the site of action of ld-IL2, we examined the effects of

SNI and ld-IL2 on the distribution of CD3+ T cells, especially the Treg cells, in the sciatic nerves and the lumbar L4 dorsal root ganglia (DRG) of female DEREG transgenic mice that express EGFP in Treg cells. There were very few CD3+ or EGFP+ T cells in the dorsal horn of the spinal cord after SNI and/or ld-IL2. In contrast, ld-IL2 significantly increased the frequency of Treg cells among total CD3+ T cells in the injured sciatic nerves but not in the uninjured nerves or the DRG at 30 days post-SNI, suggesting the injured nerve as ld-IL2's site of action. Collectively, the present study identifies Treg cell as a promising target and ld-IL2 as a potential therapy for nerve injury-induced persistent punctate and dynamic mechanical allodynia that are highly debilitating and are largely unresponsive to available treatments.

Disclosures: R. Hu: None. **J. zhang:** None. **X. liu:** None. **D. Huang:** None. **Y. Cao:** None.

Poster

P310. Neuroprotective Mechanisms: Preclinical: Other Therapeutics II

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Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P310.05

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

Support: Ministère de l'Economie et des Finances and the Région Auvergne-Rhône-Alpes [DOS0082366 and DOS0082367]

Title: Target engagement of a SCO-spondin-derived peptide ensuring neuroprotection and synaptic transmission in the CNS

Authors: *S. LEMARCHANT[1], M. SOURIOUX[1], S. AGUÉRO[2], S. HUGUES[3], M. FARINELLI[3], N. CALLIZOT[4], J. LE DOUCE[1], N. DELÉTAGE[1], R. TERREUX[2], Y. GODFRIN[1];
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Abstract: Neurodegeneration and synaptopathy are common features of CNS diseases and injuries which disrupt neural circuits and the execution of motor and cognitive functions. The subcommissural (SCO)-spondin is a brain-specific glycoprotein that strongly contributes to neuronal development during embryogenesis. The aim of this study was to investigate the effect of a SCO-spondin-derived peptide (NX210c) on neuroprotection and synaptic transmission and to identify NX210c receptors mediating such properties. Cell culture, electrophysiology and *in silico* methods were used as target engagement assays in parallel. NX210c promoted neuronal survival and prevented neurite network retraction against glutamate (glu)-induced excitotoxicity on rat cortical neurons by activating the PI3K/mTOR pathway and by disrupting apoptosis. NX210c neuroprotective effect was abolished in presence of an anti-β_1-integrin antibody (cell

death: glu = 31%, glu/NX210c = 10%, glu/NX210c/antibody = 33%) or an inhibitor of Notch signaling pathway (cell death: glu = 31%, glu/NX210c = 9%, glu/NX210c/DAPT = 23%), suggesting that β_1-integrin and Notch may represent putative receptors of NX210c (Delétage *et al.*, 2021). NX210c increased field excitatory postsynaptic potentials (fEPSP) in the somatosensory cortex after stimulation of the ventrobasal thalamus compared to vehicle-perfused brain slices (normalized fEPSP slopes at 400 µA: vehicle = 88 µA vs NX210c =119 µA). Similarly, NX210c also increased fEPSP in the hippocampal CA1 stratum radiatum after stimulation of Schaffer collaterals and commissural pathway. Evoked isolated NMDA- and AMPA-receptor excitatory postsynaptic currents (EPSC) were significantly increased in hippocampal CA1 pyramidal neurons after stimulation of Schaffer collaterals when brain slices were perfused with NX210c (amplitude of NMDA EPSC: vehicle = 53 pA vs NX210c = 102 pA). Further patch clamp experiments showed that on contrary to GluN2B, GluN2A subunit of NMDA receptors triggered the increase of EPSC in presence of NX210c. According to the results obtained using cell cultures and electrophysiology, an *in silico* approach using SUMO® and Rosetta® docking methods confirmed the strong binding of NX210c to β_1-integrin, Notch1, GluN2A subunit of NMDA receptors, and GluA1, GluA2 and GluA3 subunits of AMPA receptors. Collectively, these results show that NX210c represents an innovative drug candidate with a unique multifunctional mechanism of action to treat CNS diseases and injuries; it triggers neuroprotection through its binding to β_1-integrin and Notch receptors, and synaptic transmission through its binding to specific glutamatergic receptors.

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Poster

P310. Neuroprotective Mechanisms: Preclinical: Other Therapeutics II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P310.06

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

Support: NIH R56 DE029816

Title: Dental pulp stem cells produce higher levels of C5aR-mediated BDNF compared to bone marrow-derived mesenchymal stem cells

Authors: *M. IRFAN[1], J. KIM[2], S. RAVINDRAN[1], S. CHUNG[1];
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Abstract: Dental pulp stem cells produce higher levels of C5aR-mediated BDNF compared to bone marrow-derived mesenchymal stem cells

Muhammad Irfan, Ji Hyun Kim, Sriram Ravindran, and Seung Chung
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Abstract
A promising attempt to regenerate peripheral nerve involves the cell-based therapy that can differentiate to neurons and/or secrete trophic factors to promote nerve regeneration. Dental pulp stem cells (DPSCs) are derived from the neural crest, which also gives rise to peripheral neurons and glia. Their similarity with the neuronal cells such as the strong neuronal marker molecules and neurotransmitter receptor expression suggests that DPSCs can actively adopt in the neuronal environment and promote neural regeneration. Furthermore, DPSCs are relatively easy to obtain through a minimally invasive procedure in adults and children. We have recently demonstrated that the complement C5a system, which is an important mediator of inflammation and tissue regeneration, is activated after complement activation by lipoteichoic acid-treated pulp cells, governs the production of brain-derived nerve growth factor (BDNF). This BDNF secretion promotes neurite outgrowth towards the injury site. Here, we extend our observation to DPSCs and compare their neurogenic ability to bone marrow-derived mesenchymal stem cells (BM-MSCs). Our ELISA and immunostaining data demonstrate that C5a receptor (C5aR) blocking reduced BDNF production on DPSCs, while the treatment with C5aR agonist increased the BDNF expression suggesting C5aR`s positive regulatory role in the BDNF modulation on DPSCs. Importantly, DPSCs produced significantly higher levels of C5aR-mediated BDNF compared to BM-MSCs. This observation is consistent with our real-time qPCR data. Taken together, these data make DPSCs as an ideal stem cell source for neural regeneration.
Key Words: BDNF, Complement C5a receptor, DPSC, Inflammation, Stem cells
Conflict of Interest: None
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Disclosures: M. Irfan: None. **J. Kim:** None. **S. Ravindran:** None. **S. Chung:** None.

Poster

P310. Neuroprotective Mechanisms: Preclinical: Other Therapeutics II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P310.07

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

Support: NIH 5T32EY013934-18
NIH R01 EY020895

Title: Investigating the regulation of T148 phosphorylation on alphaA-crystallin and its impact on interactions with apoptosis-related proteins

Authors: *Z. B. SLUZALA, P. E. FORT;
Ophthalmology and Visual Sci., Univ. of Michigan, Ann Arbor, MI

Abstract: Our lab has shown that the functional and biochemical properties of the molecular chaperone protein αA-crystallin are regulated by its phosphorylation on residue T148. We previously showed that its solubility, oligomeric structure, and chaperone and neuroprotective functions are all controlled by T148 phosphorylation. Mechanistically, while we have shown that αA-crystallin regulates Bax translocation by interacting with and sequestering it into the cytoplasm, αA-crystallin's interactome and its regulation by T148 phosphorylation were still unknown. In the present study, we set to identify the kinase responsible for T148 phosphorylation and characterize αA-crystallin's interactome and its regulation by T148 phosphorylation. Since we showed that αA-crystallin is highly induced in both retinal neurons and glia we performed this study in both differentiated R28 retinal neurons and MIO-M1 Müller glial cells. Cells were transfected with plasmids encoding either 3x-FLAG-tagged wild-type (WT) or 3x-FLAG-tagged T148C αA-crystallin and exposed to "diabetes-like" stress (2h incubation with 100ng/ml TNFα and 25mM glucose) which we previously showed induced phosphorylation. Kinase identification was performed using the chemoproteomic PhAXA assay which requires a chemical crosslinker to stabilize kinase/substrate interactions, after which kinase/substrate complexes are immunoprecipitated and analyzed via western blot (WB) or liquid chromatography with tandem mass-spectrometry (LC-MS/MS). Specific complexes were observed by WB in the T148C +crosslinker condition, and CK2, one of the top candidate kinase predicted by bioinformatics, was identified by LC-MS/MS. Confirming the specificity of the kinase interaction, it could be outcompeted by incubation with excess ATP. Regarding αA-crystallin interactome, LC-MS/MS analysis of the 3x-FLAG-tagged WT pulldowns revealed 43 proteins shown through KEGG pathway analysis to be apoptosis-related, including Bax. The current study for the first time supports the prediction that CK2 can phosphorylate αA-crystallin and that αA-crystallin's interactome includes multiple apoptosis regulatory proteins in retinal neurons, consistent with the strong neuroprotective role of αA-crystallin in these cells.

Disclosures: Z.B. Sluzala: None. **P.E. Fort:** None.

Poster

P310. Neuroprotective Mechanisms: Preclinical: Other Therapeutics II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P310.08

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

Support: MSSOC Grant 3527
CIHR FDN 167270
Harley N. Hotchkiss Postdoctoral Fellowship

Title: Dual threats of CSPGs in multiple sclerosis through inhibiting oligodendrocyte repair and polarizing Th17 cells

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Abstract: In multiple sclerosis (MS), oligodendrocytes and myelin are damaged by immune responses. Remyelination failure contributes to the axonal loss and progression of disability. The failed repair process could be the consequence of ongoing toxic neuroinflammation and to inhibitors in lesions. The extracellular matrix (ECM) molecules, including members of the chondroitin sulfate proteoglycan (CSPG) family, contribute to the altered microenvironment of MS lesions. CSPGs, particularly versican V1, have been described to directly inhibit the differentiation of oligodendrocyte precursor cells (OPCs) and to prevent remyelination. Herein, we examined ECM members for their expression in lesions of MS and experimental autoimmune encephalomyelitis (EAE), a model of MS, and addressed whether these ECM molecules alter immune responses that then affect tissue repair in MS lesions.
By immunofluorescence confocal microscopy, we found increased levels in spinal cord EAE lesions of versican V1, fibronectin, thrombospondin-1 and heparan sulfate proteoglycans (HSPGs), but not versican V2 or aggrecan. Versican V1 deposition was confirmed in active MS lesions. In culture, a mixed CSPG preparation and purified versican V1, but not other ECM molecules tested, shifted T cell differentiation toward Th17 cells, which were then inhibitory for OPC differentiation. CSPG-polarized Th17 cells also killed OPCs. To inhibit CSPG synthesis in vivo, EAE mice were injected with difluorosamine (peracetylated,4,4-difluoro-N-acetylglucosamine) starting at the peak of clinical disease severity. Difluorosamine-treated mice has reduced injury-enhanced versican content in spinal cord lesions correspondent with lower frequency of Th17 cells. To study the effect of difluorosamine on remyelination during EAE, NG2-Cre:Tau-mGFP mice were used with GFP expression localized to newly formed oligodendrocytes and myelin. Drug was initiated from peak clinical
severity. We found that difluorosamine reduced levels of versican V1 in well-formed EAE lesions resulting in higher number of new oligodendrocytes and myelin sheets around and within lesions. These results highlight lesion-elevated CSPGs, especially versican V1, in directly inhibiting OPCs and indirectly in shaping T cell responses which then impact remyelination outcomes. We propose CSPG-lowering drugs as dual pronged repair therapeutics that directly affect OPCs, and that indirectly antagonize Th17 roles in inflammation and oligodendrocyte injury.

Disclosures: S. Ghorbanigazar: None. **W. Yong:** None.

Poster

P315. Neuroinflammation: Oligodendrocytes, Pericytes, and Others

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P315.01

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

Support: Sanofi

Title: Srebp2 signaling is essential for emopamil binding protein inhibitor-induced oligodendrocyte precursor cell differentiation

Authors: *G. SHENG[1], C. ZHU[1], Y. HUANG[1], J. FARLEY[1], J. GANS[1], S. MADDEN[1], A. BYRNE[1], L. GUO[1], X. CHAMLING[2], D. ZACK[2], K. RADZWILL1[1], G. MERRIMAN[1], G. GAO[1], T. TRELEAVEN[1], C. GARRON[1], L. CHAI[1], S. GIERA[1], B. ZHANG[1], D. RAJPAL[1], C. PEDRAZA[1], P. SARDI[1], J. DODGE[1];
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Abstract: Immunotherapies significantly reduce CNS lesion formation and relapse rate in Multiple Sclerosis (MS) patients; however, they largely fail to prevent the accumulation of permanent disability that arises from axonal and neuronal damage, particularly during the progressive phase of the disease. Thus, therapeutic strategies that stimulate remyelination to promote neuroprotection are being explored for the treatment of MS. Pharmacological enhancement of myelination through the maturation of endogenous oligodendrocyte precursor cells (OPC) is responsive to several stimuli, including compounds that induce the accumulation of intermediate sterols. Specifically, inhibition of sterol isomerase emopamil binding protein (EBP), which catalyzes the conversion of zymosterol and zymostenol to dehydrolathosterol and lathosterol in the cholesterol biosynthesis pathway, effectively promotes oligodendrocyte precursor cell differentiation and remyelination in preclinical models of MS. However, the mechanism underlying the effect of EBPi on OPC maturation remains unknown. Here, we treated rat OPCs with EBP inhibitors or
triiodothyronine (T3) for 72 hours and then performed RNA-Sequencing. Differential expression profiles were found for both EBPi and T3. Interestingly, a subset of genes regulated by sterol regulatory element binding protein 2 (SREBP2) were significantly upregulated by EBPi but not by T3. Similarly, in single-cell RNA sequencing of the human embryonic stem cell-derived OPCs we found that SREBP2 was also activated by the EBPi. Repeated experiments showed a consistent upregulation of the SREBP2 signaling pathway by EBPi. Lastly, we found that inactivating SREBP2 signaling using PF-429242, an inhibitor of site-1 protease (S1P) which cleaves the SREBP2 precursor into its mature form, prevented EBPi induced OPC differentiation and the expression of myelin-associated genes. These results suggest that activation of the SREBP2 signaling pathway plays a crtical role in EBPi induced OPC differentiation and myelin formation**.**

Disclosures: G. Sheng: A. Employment/Salary (full or part-time):; Sanofi. **C. Zhu:** A. Employment/Salary (full or part-time):; Sanofi. **Y. Huang:** A. Employment/Salary (full or part-time):; Sanofi. **J. Farley:** None. **J. Gans:** A. Employment/Salary (full or part-time):; Sanofi. **S. Madden:** A. Employment/Salary (full or part-time):; Sanofi. **A. Byrne:** A. Employment/Salary (full or part-time):; Sanofi. **L. Guo:** A. Employment/Salary (full or part-time):; Sanofi. **X. Chamling:** B. Contracted Research/Research Grant (principal investigator for a drug study,

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Poster

P315. Neuroinflammation: Oligodendrocytes, Pericytes, and Others

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P315.02

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

Support: TUBITAK 1001 (116S552)
TUBITAK 2236 (119C018)

Title: Pericytes play a protective role during neuroinflammation

Authors: *D. ATAK, A. B. GÖKYÜZÜ, E. YILDIZ, E. ÖZKAN, M. YOUSEFI, A. B. KIZILIRMAK, Y. GÜRSOY-ÖZDEMIR, A. VURAL;
Koç Univ., Istanbul, Turkey

Abstract: Background: Pericytes play a key role in blood-brain-barrier (BBB) maturation and maintenance of immune homeostasis in the central nervous system (CNS). Ablation of pericytes in developing mice causes vascular abnormalities, BBB leakage and, increased infiltration of leukocytes to the CNS in later life both in steady-state and pathological conditions. However, the effect of pericyte ablation during neuroinflammation in the presence of a fully mature BBB is unknown. **Aim:** In this study, we first aimed to generate a tamoxifen-inducible pericyte ablation model in adult mice. Then, we investigated the effect of pericyte loss on neuroinflammation in an experimental allergic encephalomyelitis (EAE) model. **Methods:** PDGFRβ-PA2-CreER+/- mice (JAX Mice, 030201) were crossed with Rosa26-DTA176+/+ mice (JAX Mice, 010527). After genotyping, 9-10 weeks old PDGFRβ-Cre+/DTA176+ (Cre+) mice were injected with 0.1mg/g tamoxifen (TAM) for two, three, or five consecutive days to induce the expression of diphtheria toxin in PDGFRβ+ pericytes. PDGFRβ-Cre-/DTA176+ (Cre-) mice were used as

controls. After 15 days, animals were sacrificed to assess pericyte coverage and numbers, and PDGFRβ gene expression in the cortex and spinal cord. After analysis, 2X TAM injection model was selected for EAE studies. MOG35-55 (Hooke Labs, EK2110) was used to induce EAE 15 days after TAM injection. Cre- and wild-type C57BL/6 mice were used as controls. Weight change and clinical scores were monitored for 28 days. Analysis was done by repeated-measures 2-way ANOVA multiple comparison test. **Results:** There was a 75% (+-SEM=0.9192) decrease in pericyte coverage in the cortex ($p<0.01$) and a 40% (+-SEM=2.385) decrease in the spinal cord ($p<0.01$) after 2X TAM injection (n=7). Similarly, Pdgfrβ gene expression was decreased to 55% (+-SEM=2.230) in the cortex ($p=0.017$) and 59% (+-SEM=4.511) in the spinal cord ($p=0.048$). During EAE, pericyte-ablated mice (n=7) had a more severe clinical score after Day 20 ($p=0.0086$, 0.0036, 0.0054, 0.07,0.041). Pericyte ablated mice lost more weight than the wild type mice ($p=0.0020$, 0.0101, 0.0039, 0.0064, 0.0181, 0.0091, 0.0006 from Day16 to 28). In addition to the spinal cord lesions, inflammatory foci were also detected in the periventricular region in the brain of the pericyte ablated mice but not in control mice. **Comment:** We have generated and characterized a TAM inducible pericyte ablation model in adult mice that does not require injection of a toxin. By using this model, pericytes were shown to play an important role during the resolution of inflammation.

Disclosures: D. Atak: None. **A.B. Gökyüzü:** None. **E. Yildiz:** None. **E. Özkan:** None. **M. Yousefi:** None. **A.B. Kizilirmak:** None. **Y. GÜRSOY-ÖZDEMiR:** None. **A. Vural:** None.

Poster

P315. Neuroinflammation: Oligodendrocytes, Pericytes, and Others

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P315.03

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

Support: Race to Erase MS

Title: Differential regulation of metabolic pathways in oligodendrocyte precursor cells in response to distinct stimuli

Authors: *J. LEE, M. KORNBERG;
Johns Hopkins Univ., Baltimore, MD

Abstract: Multiple sclerosis (MS) is an autoimmune neurodegenerative disease with features of demyelination by self-reactive T cells and limited remyelination as oligodendrocytes that produce myelin are damaged. In MS lesions, the differentiation of oligodendrocyte progenitor cells (OPC) to oligodendrocytes is blocked, resulting in a lack of mature oligodendrocytes to repair myelin. The mechanisms underlying this blockage of OPC differentiation are poorly understood. Therefore, it is critical to investigate the mechanisms of OPC differentiation to

promote remyelination and develop regenerative treatments to complement current therapies that target the immune system and rely on anti-inflammatory effects. Interestingly, inflammatory cytokines have also been shown to inhibit OPC differentiation and result in a different phenotype featuring characteristics similar to activated immune cells. The metabolic balance between glycolysis and oxidative phosphorylation critically regulates immune cell fate; further, a metabolic switch is observed in stem cells, which rely on a high glycolysis rate to preserve their self-renewal capacity. These findings reveal a new function of OPCs in inflammation and suggest the possibility of metabolic regulation of OPC fate, with metabolic reprogramming serving as a checkpoint between pro-inflammatory and remyelinating OPC phenotypes. In this study, we investigated the metabolic programming of OPCs in response to various factors. We exposed OPCs to distinct stimuli including proliferating (PDGF-aa), differentiation promoting (T3), and inflammatory (IFN-γ) signals. We then measured the extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) to analyze real-time ATP production rate. OPCs treated with T3 had a decrease in overall ATP production when compared to cells treated with PDGF-aa. IFN-γ treatment also decreased overall ATP production, but with a higher dependency of oxidative phosphorylation for ATP production. We plan to evaluate glycolytic programming of OPCs in distinct contexts of inflammation or remyelination *in vivo*. Further on, we will perform untargeted metabolomics identify metabolic pathways and metabolites that regulate inflammatory vs. remyelinating OPC fates. We will correlate metabolomics analysis to existing RNA-seq.

Disclosures: J. Lee: None. **M. Kornberg:** None.

Poster

P315. Neuroinflammation: Oligodendrocytes, Pericytes, and Others

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P315.04

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

Support: NIH Grant MH119346
Charles A. Dana Foundation
Adelson Medical Research Foundation

Title: Integrin-αM/cd11b inhibits β-glucan elicited regeneration of mammalian optic nerve axons

Authors: *R. PASSINO[1], X.-F. ZHAO[1], C. JOHNSON[1], L. HUFFMAN[2], M. FINNERAN[2], R. KAWAGUCHI[3], D. H. GESCHWIND[3], R. J. GIGER[1];
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Abstract: The immune system and the nervous system are in constant dialogue, and this interaction is particularly intense following nervous system injury. Intra-ocular injection of polymeric β [1-3]-glucose (β-glucan) triggers a strong immune response in the eye. When combined with retro-orbital optic nerve crush injury (ONC), β-glucan promotes lengthy regeneration of severed retinal ganglion cell (RGC) axons. In marked contrast, intra-ocular lipopolysaccaride triggers an immune response that does not support RGC axon regeneration. For an in-depth analysis of the β-glucan triggered immune response, we micro-dissected retinas and optic nerves from mice 3 days after ONC and intra-ocular injection of β-glucan or saline (PBS). Innate immune cells were isolated by immunopanning and subjected to single cell RNA-sequencing. A comparative analysis revealed that the retinal/intra-ocular immune milieu in β-glucan treated mice is dominated by neutrophils, monocytes/macrophages, and few microglia. In the injured optic nerve, microglia greatly outnumber macrophages and seven different microglia subpopulations were identified. Pharmacological ablation of microglia with the CSF1R inhibitor PLX56622 attenuated β-glucan elicited optic nerve regeneration, suggesting that microglia are necessary for immune-mediated axon regeneration. Differential gene expression and pathway analysis of scRNA-seq data under regenerating and non-regenerating conditions identified complement activation as one of the top hits. The complement receptor 3 (CR3) is composed of two subunits CD11b/(integrin αM) and CD18/(integrin β2). Because Integrin αM directly binds to β-glucan, we subjected *Itgam*-/- mice to intra-ocular β-glucan injection and ONC. Surprisingly, optic nerves from *Itgam*-/- mice showed significant increased axon regeneration when compared to parallel processed WT nerves. Increased RGC regeneration in *Itgam*-/- mice was blocked when combined with PLX5622 treatment. The complement component C3 is strongly upregulated in the retina of β-glucan injected mice. However, the increased RGC regeneration observed in *Itgam*-/- mice is not due to disruption of the CR3 receptor, since *C3*-/- mice failed to mimic the RGC regeneration phenotype observed in *Itgam*-/- mice. Ongoing experiments are focused on exploring the mechanism(s) that underlie the increased RGC axon regeneration observed in *Itgam*-/- mice.

Disclosures: R. Passino: None. **X. Zhao:** None. **C. Johnson:** None. **L. Huffman:** None. **M. Finneran:** None. **R. Kawaguchi:** None. **D.H. Geschwind:** None. **R.J. Giger:** None.

Poster

P315. Neuroinflammation: Oligodendrocytes, Pericytes, and Others

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P315.05

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

Support: NIH Grant - NINDS R01 NS105385
NIH Grant - NINDS F31 NS118881

Title: The Regulatory Role of TAM Receptors in Experimental Autoimmune Encephalomyelitis

Authors: *A. MUNIE[1], B. SEGAL[2];
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Abstract: Multiple Sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS), believed to be autoimmune in etiology. Myeloid cells comprise the majority of inflammatory cells in MS lesions and may play both pathogenic and regulatory roles during disease evolution. Studies in experimental autoimmune encephalomyelitis (EAE), a murine model of MS, indicate that activation of TAM family of receptor tyrosine kinases (Tyro3, Axl, Mer) on myeloid cells curbs neuroinflammation and promotes neuroprotection, thereby improving clinical outcomes. However, the mechanism of action of TAM receptors in this context is not fully understood. We found that Axl and MerTK are upregulated on a subset of alternatively activated Arg-1+ macrophages and monocyte-derived dendritic cells, that accumulate in the brain and spinal cord from the peak to late stages of EAE. EAE typically presents as an ascending paralysis associated with spinal cord lesions. Treatment with a TAM antagonist (LDC1267) converted the clinical manifestation of EAE to an atypical from, characterized by ataxia and hindbrain lesions. Collectively, our data suggest that the dominant effect of TAM receptor signaling in CNS myeloid cells is to selectively protect the brain against inflammatory infiltration. These findings reflect the heterogeneity of neuroinflammatory pathways across CNS compartments, which might explain the diversity of MS with respect to clinical course and therapeutic responsiveness.

Disclosures: A. Munie: None. **B. Segal:** None.

Poster

P315. Neuroinflammation: Oligodendrocytes, Pericytes, and Others

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Program #/Poster #: P315.06

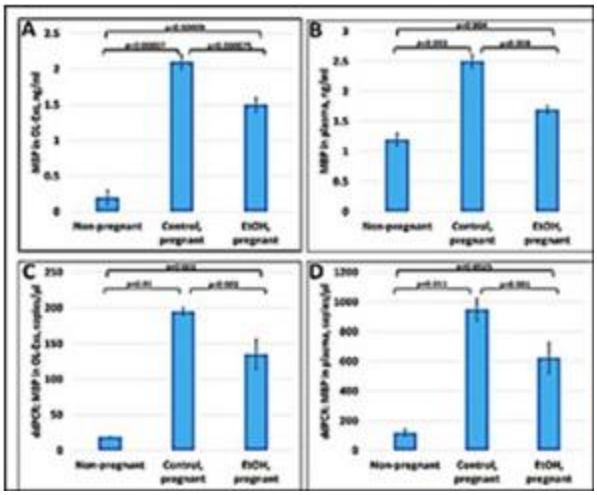
Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

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Title: Exosomal lipid biomarkers of oligodendrocyte pathology during fetal alcohol exposure

Authors: *M. E. SELZER[1,2], A. DARBINYAN[3], N. MERABOVA[1,4], E. C. SPARKS[1], T. TATEVOSIAN-GELLER[1], L. GOETZL[5], N. DARBINIAN[1];
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Abstract: Introduction: One structural hallmark of Fetal Alcohol Syndrome (FAS) is a massive loss of neurons and oligodendrocytes (OLs). Dysregulation of lipids in myelin leading to apoptosis of oligodendrocyte precursor cells (OPCs) and altered OL differentiation has been shown in FAS rodent models but there are significant gaps in our understanding of the FAS-specific mechanisms of OL lipid loss and enhanced dysmyelination in the human fetus. We recently demonstrated an EtOH-dependent decrease in myelin basic protein (MBP, a marker for mature OLs), increased markers for OPC, increased in caspase-3 activity, and increased TNF-α in the human fetal brain early in gestation. Free fatty acid (FFA) effects are mediated *via* specific G protein-coupled receptors (FFAR), influencing OL development and myelination. We hypothesize that FAS is associated with a deficiency in ω-3 FFA, and that OL-derived exosomes (OL-Es), which can be isolated from maternal serum, carry FFA biomarkers for FAS. **Methods**: Blood samples were collected from EtOH-exposed and control pregnant women, stratified by gestational age, gender, and race according to an IRB-approved protocol. OL-Es were isolated from 250 μL of maternal blood, using MBP. EtOH-associated dysmyelination was assessed. OL-E mRNA was assayed by ddPCR for OL markers and FFAR expression. OL-E MBP and FFAR protein levels were quantified by ELISA (normalized to CD81) and flow cytometry assays. **Results**: Downregulation of FFAR was greatest in EtOH-exposed cases (n=6). Statistically significant downregulation of the MBP mRNA and protein levels also was found in EtOH-exposed OL-Es, but not in non-pregnant women. **Conclusion**: Non-invasive assessment of fetal OL-E biomarkers in clinical studies offers an opportunity to confirm pre-clinical findings. Future studies should focus on the correlation between OL-E markers and clinical phenotype/outcomes in FAS offspring and could further lead to clinical trials of dietary corrections of lipid abnormalities (*e.g.*, dietary ω-3 supplementation), to reduce dysmyelination in at-risk fetuses and in children with FAS.



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Poster

P315. Neuroinflammation: Oligodendrocytes, Pericytes, and Others

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Program #/Poster #: P315.07

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

Support: NIH NCI 5R01CA194924
NIH NINDS R01NS092388
NIH NIGMS 5U54GM104942-03

Title: Social Enrichment Ameliorates Chemotherapy Induced Peripheral Immune Cell Trafficking

Authors: W. H. WALKER, II[1], J. R. BUMGARNER[1], J. A. LIU[1], O. H. MELÉNDEZ-FERNÁNDEZ[1], J. C. WALTON[1], R. J. NELSON[1], A. C. DEVRIES[1,2,3];
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Abstract: Chemotherapy is a mainstay in cancer treatment. It is estimated that as many as 650,000 cancer patients receive chemotherapy in an outpatient setting each year. Although chemotherapy has greatly increased survival rates and has superior anti-tumor efficacy, it is not without some detrimental side effects. Patients receiving chemotherapy commonly display somatic symptoms such as nausea, vomiting, diarrhea, neutropenia, and others. Furthermore, patients receiving chemotherapy commonly demonstrate non-somatic symptoms such as fatigue, depression, anxiety, and impaired cognitive function. Notably, the amount of social support prior

to cancer treatment predicts the levels of cognitive dysfunction and depressive symptoms in cancer survivors. Previous work for our lab has demonstrated the ability of social enrichment to attenuate chemotherapy induced neuroinflammation and affective behavior via oxytocin signaling in mice. However, the precise mechanism of chemotherapy-induced neuroinflammation and subsequent attenuation by social enrichment remains unknown. We hypothesized that chemotherapy increases peripheral immune cell trafficking within the CNS leading to neuroinflammation. Female Balb/C mice were randomly assigned to treatment groups and placed into their assigned housing (single or triad housing). One week later, mice received their first injection of vehicle (0.9% saline) or chemotherapeutic cocktail (9 mg/kg doxorubicin and 90 mg/kg cyclophosphamide). Fourteen days later mice received their second injection of vehicle or chemotherapeutic cocktail. One week following the second injection, mice were euthanized via transcardial perfusion, brains were extracted, and immune cells were isolated using a Percoll gradient and quantified via flow cytometry. Similar to our previous experiments, we did not observe neither an expansion of the microglia population ($CD11b^{+}$, $CD45^{intermediate/low}$, $Ly6G^{-}$) nor increased activation of microglia ($MHCII^{+}$) measured by flow cytometry following chemotherapy administration. However, singly housed mice receiving chemotherapy increased monocyte ($CD11b^{+}$, $CD45^{high}$, $Ly6C^{+}$, $Ly6G^{-}$) and neutrophil ($CD11b^{+}$, $CD45^{high}$, $Ly6C^{-}$, $Ly6G^{+}$) trafficking. Notably, social enrichment attenuated the increased neutrophil trafficking and prevented increased monocyte trafficking. These data are the first to demonstrated altered immune cell trafficking within the CNS following chemotherapy administration and provide further support for the potential beneficial effects of social enrichment.

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Poster

P315. Neuroinflammation: Oligodendrocytes, Pericytes, and Others

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Program #/Poster #: P315.08

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

Title: Cell Cycle Inhibitor p21 Expression Decrease in PPMS Cerebral Organoids Leads to Exhaustion of The Progenitor Pool Independently of Apoptosis Pathway

Authors: T. EDWARDS, E. CHEN, *N. DAVIAUD, S. A. SADIQ;
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Abstract: Multiple sclerosis (MS) is an auto-immune neurological disorder characterized by inflammation, demyelination, and neural degeneration. The origin and evolution of MS are still poorly understood because of lack of animal models and relative inaccessibility to human brain tissue. Cerebral organoids represent an interesting tool to study neurological disorders as they

recapitulate early human neurodevelopment, including the generation, proliferation and differentiation of neural progenitors into glial cell and neurons.The purpose of this work was to use patient with MS induced pluripotent stem cells derived cerebral organoids to study the genetic components of MS. This model was used to analyze the human neural stem cell population in control and MS organoids after 42 days in vitro.The cell cycle inhibitor p21 is essential for the maintenance of stem cell quiescence and self-renewal. Immunostainings showed a high expression of p21 in the ventricular zone of control organoids. This expression was highly reduced in PPMS organoids compared to control and other subtypes of MS. Further analysis of the stem cells revealed that this decrease of p21 was associated with a significant reduction of the expression of the stem cell marker SOX2 and of the proliferation marker Ki67 in PPMS organoids compared to control, indicating a smaller stem cell pool and a reduced proliferation rate respectively. To explore if p21 expression in organoids is involved in the DNA damage and apoptosis pathway, immunostainings for γH2AX, p53, and CC3 were performed. Low to no γH2AX-positive cells were found in control and PPMS organoids, indicating low DNA damage. No difference was observed between the two conditions. p53 was detected in the ventricular zone/subventricular zone in both control and PPMS organoids, but there was no difference in p53 expression between the two conditions. A few CC3+ cells were detected in organoids, particularly localized in the upper cortical layers, with low to no positive cells in the stem cell population. No difference in CC3 expression was detected in PPMS compared to control organoids, indicating that there were no or low involvement of the apoptosis pathway in MS samples. Our data indicates that the reduced p21 expression in PPMS is independent of the p53/apoptosis pathway and is related to cell cycle only, leading to an exhaustion of the progenitor pool. Patient derived cerebral organoids may be a viable human model to study MS pathogenesis.

Disclosures: T. Edwards: None. **E. Chen:** None. **N. Daviaud:** None. **S.A. Sadiq:** None.

Poster

P320. Neuroinflammation: HIV and Infections, and Elicit Drugs

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Program #/Poster #: P320.01

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

Support: NIH Grant R01DA052851
NIH Grant R00 DA039791

Title: Tat and gp120 promote microglial activation that can be ameliorated by corticosterone in a dose- and time-dependent manner: Implications for neuroHIV-like behavior

Authors: *E. MOSS[1], C. WORTH[2], F. MAHDI[1], J. PAIRS[1];
[1]Biomolecular Sci., [2]Chem. and Biochem., The Univ. of Mississippi, Oxford, MS

Abstract: The immunological effects of human immunodeficiency virus (HIV) infection are appreciated, but less well understood are its effects on the central nervous system (CNS). Combinatorial antiretroviral therapies (cART) attenuate the effects of HIV peripherally; however, approximately half of all cART-treated people with HIV contend with neurocognitive and neuropsychiatric complications referred to as "neuroHIV." The mechanisms of neuroHIV are not known, but likely involve the presence of neurotoxic HIV proteins in the CNS. In particular, the HIV virotoxins, trans-activator of transcription (Tat) and glycoprotein 120 (gp120), promote neuroinflammation via the activation of microglia. In search of novel therapeutics, we and others have found steroids to ameliorate the direct effects HIV virotoxins. We hypothesized that Tat and gp120 would activate microglia in cell culture, increase anxiety-like behavior in mice, and that steroid-based interventions would ameliorate these effects. In a human microglial cell line (HMC3) exposure to Tat (50 ng/mL) or gp120 (500 pM) increased the proportion of cells with a phagocytic morphology and reduced the proportion of resting cells when observed 6 h or 24 h later (but not 2 h later). The stress hormone, corticosterone, attenuated these effects when administered at 100 nM (but not 32 or 320 nM). Notably, the pre-glucocorticoid neurosteroid, TH-DOC, also demonstrated some capacity to attenuate Tat- (but not gp120) mediated microglial activation. When assessed i*n vivo*, conditional expression of Tat in transgenic mice increased anxiety-like behavior in an open field and this was exacerbated by co-infusion of gp120 (100 pM, bilateral intracerebroventricular). Steroid-based therapeutics may offset neuroinflammatory drivers of CNS dysfunction. In particular, glucocorticoid receptors on microglia may mediate Tat- and gp120-related activation, whereas neurosteroid targets (such as $GABA_A$ receptors) may be more involved in Tat-related activation alone. These data support the notion that CNS exposure to Tat and gp120 can exert additivity to promote neuroHIV-like behavior.

Disclosures: E. Moss: None. **C. Worth:** None. **F. Mahdi:** None. **J. Pairs:** None.

Poster

P320. Neuroinflammation: HIV and Infections, and Elicit Drugs

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

Support: R00 DA039791
R01 DA052851
GSC 001408

Title: Neurosteroids influence HIV-1 Tat-mediated HPA dysregulation and anxiety-like behavior

Authors: *M. SALAHUDDIN[1], F. MAHDI[2], J. J. PARIS[3];
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Abstract: Background: Human immunodeficiency virus (HIV) is associated with comorbid affective and stress-sensitive neuropsychiatric disorders (collectively referred to as neuroHIV) that afflict ~50% of infected individuals. Moreover, approximately ~46% of HIV patients show dysfunction of the hypothalamic-pituitary-adrenal (HPA) stress axis, but the mechanisms are not known. One factor that may contribute to HPA dysfunction is actions of the neurotoxic HIV-1 regulatory protein, trans-activator of transcription (Tat). Our prior studies in male mice revealed HIV-1 Tat to promote psychomotor and anxiety-like behavior. These behavioral effects occurred concurrent with an elevation of basal corticosterone and adrenal insufficiency in response to natural stressor or pharmacological blockade of HPA feedback, recapitulating the clinical endophenotype. The HPA axis, which mediates the neuroendocrine response to stress is tightly regulated by the GABAergic signaling and deficits of GABAergic signaling may reciprocally contribute to neuroendocrine dysfunction, conferring vulnerability to stress-sensitive disorders. Given that neurosteroids are potent allosteric modulators of $GABA_A$ receptor, enhancing neurosteroidogenesis may ameliorate HPA-related dysfunction in the context of HIV. Hypothesis: We hypothesized that the central infusion of the 18 kDa translocator protein agonist, FGIN-1-27, will boost neurosteroidogenesis and curtail Tat-induced HPA activation and associated behavioral deficits. Methods: Transgenic mice that expressed the Tat_{1-86} protein [Tat(+)] or not [Tat(-)] were administered either vehicle or FGIN-1-27 (5 µg/µL) directly to the lateral ventricle via an ALZET osmotic minipump (7 days) and behaviorally-tested in an open field and light-dark transition task. Results: In male mice, FGIN-1-27 administration enhanced circulating corticosterone concurrent with a decrease in anxiety-like behavior demonstrated by reduced time spent in the light zone and an increased latency to enter the dark zone in the light-dark transition test in mice. These data provide proof-of-principle that enhancing neurosteroidogenesis in the central nervous system can influence the HPA axis and reduce anxiety-like behavior. Additional studies will further assess the capacity for FGIN-1-27 to ameliorate Tat-promoted HPA deficits and related affective dysfunction. Conclusion:Therapeutic strategies that promote neurosteroidogenesis may improve HIV-related stress-sensitive disorders.

Disclosures: M. Salahuddin: None. **F. Mahdi:** None. **J.J. Paris:** None.

Poster

P320. Neuroinflammation: HIV and Infections, and Elicit Drugs

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Program #/Poster #: P320.03

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

Support: NIH Grant R00 DA039791
NIH Grant R01 DA052851
The University of Mississippi School of Pharmacy

Title: Hiv-1 tat influences morphine reward and voluntary oral morphine consumption among ovariectomized female mice

Authors: *A. QRAREYA[1], M. SALAHUDDIN[1], F. MAHDI[1], T. VUJANVIC[1], P. SERRANO[2], J. PARIS[1];
[1]Univ. of Mississippi, University, MS; [2]Hunter Col., New York, NY

Abstract: In the era of combinatorial antiretroviral therapy, people with human immunodeficiency virus (HIV) are still at risk for chronic pain (~25-90%). As a consequence, opioids are clinically prescribed to a greater degree in the HIV+ population (~21-53%). HIV-infected individuals may be more vulnerable to develop opioid tolerance and substance abuse exacerbates HIV-associated neurocognitive disorders. While the mechanisms that underlie HIV interactions with opioids are not well-understood, we and others have found the HIV-1 regulatory protein, trans-activator of transcription (Tat), to potentiate the rewarding properties of drugs of abuse in male mice. We hypothesized that Tat expression would potentiate morphine's rewarding and reinforcing effects in ovariectomized (OVX) female mice that conditionally-expressed Tat in a doxycycline-dependent manner. As expected, inducing Tat expression significantly potentiated morphine-conditioned place preference (CPP) among OVX female Tat-transgenic mice. However, when mice were allowed to voluntarily self-administer a control quinine solution (2 mg/kg, PO) or escalating doses of morphine (0.6, 1.2, 2 mg/kg, PO) for 14 days, Tat-exposed mice consumed significantly less morphine (mg/kg body weight) than did control mice. All mice self-administrated more morphine than quinine. Naloxone-induced withdrawal behavior confirmed that mice had reached morphine-dependence. In a follow-up experiment, mice were exposed to quinine or escalating doses of morphine for 21 days. All mice escalated morphine consumption to a greater degree than in the 14-day experiment; however, Tat-exposed mice continued to voluntarily self-administer less morphine than control mice. Together, data obtained in the CPP paradigm may indicate an increase in the rewarding valence of morphine among Tat-exposed females, thus requiring less self-administration in the voluntary oral consumption task order to achieve an optimal hedonic effect. Differences in the neurocircuitry of brain regions involved in drug of abuse reward and reinforcement will be sought. Thus, these data demonstrate that HIV-1 Tat protein enhances the rewarding properties of morphine while reducing the demonstration of reinforcing behavior.

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Poster

P320. Neuroinflammation: HIV and Infections, and Elicit Drugs

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

Support: NIH R01DA044939

Title: Fronto-cortical neurovascular damage induced by morphine and HIV-1 Tat resembles accelerated brain aging

Authors: *M. OHENE-NYAKO[1], Y. HAHN[2], S. R. NASS[3], P. E. KNAPP[4], K. F. HAUSER[5]; [2]Anat. & Neurobio., [3]Dept. of Pharmacol. and Toxicology, [4]Dept Anat & Neurobio., [5]Pharmacol. & Toxicology, [1]Virginia Commonwealth Univ., Richmond, VA

Abstract: Fronto-cortical neurovascular damage induced by morphine and HIV-1 Tat resembles accelerated brain aging.
Authors: Michael Ohene-Nyako, Yun K. Hahn, Sara R. Nass, Pamela E. Knapp, and Kurt F. Hauser Fronto-cortical neurodegeneration occurs in HIV-infected individuals. In age-associated neurocognitive disorders, such pathology indicates the presence of pathologic protein aggregates, small vessel disease, and neuroaxonal degeneration. Opiate abuse is prevalent in the HIV-infected population; however, it is unknown if the comorbidity converges to enhance fronto-cortical age-related pathologic changes. We evaluated (i) vascular integrity using levels of claudin-5 (cldn5) and albumin (Alb), (ii) pathologic protein aggregates, and (iii) neuroaxonal damage by examining hyperphosphorylated tau load (p-tau), beta-amyloid (Aβ) levels, oligodendroglial (OLG) numbers, and neurofilament light chain (NFL) levels in the prefrontal cortex (PFC) of control and inducible HIV-1 Tat transgenic male mice that received morphine (10–40 mg/kg, increasing by 10 mg/kg/b.i.d., s.c. weekly) for 6 weeks. Levels of cldn5, Alb, Aβ, and NFL were evaluated by immunoblotting. Sub-anatomical Cldn5 expression, Alb leakage, OLGs, and p-tau load were determined by immunofluorescence assays in the anterior cingulate (ACC) and primary motor (M1) cortices. Tat decreased cldn5 and increased Alb levels in the PFC. Morphine exacerbated the Tat effects on cldn5 and Alb. Tat, and to a lesser extent morphine exposure, increased levels of NFL, Aβ peptide, and oligomeric forms. Enhanced NFL and Aβ pathology were observed in the comorbid brain. Immunofluorescence findings revealed that Tat and morphine independently dysregulated cldn5 and enhanced Alb leakage in the ACC. Morphine, but not Tat, altered Cldn5 and Alb immunofluorescence in the M1 cortex. The proportion of ACC p-tau-immunoreactive cells was increased by Tat irrespective of morphine treatment, but the opposite was observed in the M1. Morphine decreased CC1-stained OLG numbers in the ACC irrespective of genotype. No OLG reductions were observed in the M1. Neuroaxonal injury and small vessel disease in the M1 and ACC are associated with motor and cognitive dysfunction in Alzheimer's disease. Thus, our results may predict an accelerated risk for developing similar age-associated disorders in opiate abusing, HIV-infected individuals.
1

Disclosures: M. Ohene-Nyako: None. **Y. Hahn:** None. **S.R. Nass:** None. **P.E. Knapp:** None. **K.F. Hauser:** None.

Poster

P320. Neuroinflammation: HIV and Infections, and Elicit Drugs

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Support: NIDA F32 DA053163
NIDA R01 DA018633
NIDA R01 DA045588

Title: Contrasting effects of HIV-1 Tat and morphine on depressive-like behaviors are associated with inflammation and neurodegeneration within prefrontal cortico-amygdalar circuits

Authors: *S. R. NASS[1], M. OHENE-NYAKO[1], V. D. MCLANE[1], Y. K. HAHN[2], P. E. KNAPP[1,2,3], K. F. HAUSER[1,2,3];
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Abstract: HIV-1 trans-activator of transcription (HIV-1 Tat) can be expressed in the CNS of persons infected with HIV (PWH) resulting in emotional deficits, despite antiretroviral therapy, and these deficits can be further exacerbated by opioid use. Within prefrontal cortico-amygdalar (PFC-amygdala) circuits, inflammation and neurodegeneration can lead to depression and anxiety. To explore HIV-1 Tat- and morphine-induced emotionality deficits, Tat(+) and Tat(-) male mice were exposed to HIV-1 Tat for 8 weeks and administered saline or ramping doses of morphine twice daily (s.c.) during the last 2 weeks of HIV-1 Tat exposure. Mice were behaviorally tested in a battery of depressive-like behaviors 4 h after morphine administration to limit opioid-induced locomotor effects. In the novelty suppressed feeding test, morphine increased eating behavior, whereas in the novelty induced hypophagia test, Tat decreased sucrose intake, indicating that morphine and Tat differentially alter anhedonia in response to palatable and non-palatable food stimuli. Mice were also test in assays of daily living to model apathy. In home cages, Tat also decreased nesting behavior, whereas morphine decreased burrowing. Together, these data suggest that HIV-1 Tat and morphine differentially induce depressive-like behavior. Although innate immune tolerance appeared to develop within the PFC, Tat(+) mice administered morphine exhibited increased depressive-like behavior that correlated positively with PFC levels of CXCL1 in the forced swim test; whereas previous data indicated increased mobility in the forced swim test after Tat exposure alone correlated negatively with PFC expression of CXCL1. Interestingly, in Tat(+) mice administered morphine increased levels of TNFα, IL-3, and IL-10 also positively correlated with increased response to food stimuli. Tat decreased dendritic spine density on pyramidal neurons in layer V of the anterior cingulate region of the prefrontal cortex and the basolateral amygdala (BLA), but not the medium spiny-

type neurons within the central nucleus of the amygdala (CEA). Tat also increased amyloid-β (Aβ) levels within the amygdala. Together, our findings suggest that HIV-1 Tat and morphine differentially induce depressive-like behavior and are associated with Tat-induced neuronal damage within the prefrontal-amygdalar circuits.

Disclosures: S.R. Nass: None. **M. Ohene-Nyako:** None. **V.D. McLane:** None. **Y.K. Hahn:** None. **P.E. Knapp:** None. **K.F. Hauser:** None.

Poster

P320. Neuroinflammation: HIV and Infections, and Elicit Drugs

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Support: NIH Grant T32AI007172
NIH Grant R01NS052632

Title: Cxcr6 contributes to neuroinflammation by maintaining tissue resident memory cd8+ cells in the cns in a viral recovery model

Authors: *S. F. ROSEN, A. SOUNG, W. YANG, S. AI, M. KANMOGNE, V. DAVE, J. A. MAGEE, R. S. KLEIN;
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Abstract: $CD8^+$ T cells are key players in pathogen elimination and viral clearance after central nervous system (CNS) infection. However, antiviral T cells can persist in the CNS for years after recovery from neurotropic viral infections. While T cells are important initially for viral clearance, their persistence in the CNS can lead to chronic microglial activation and long-term cognitive sequelae and neuronal damage. Published data from our lab has shown that $CD8^+$ T cell derived IFN-γ signaling in microglia causes persistent microglial activation in the CNS, and contributes to learning and memory deficits after recovery from viral infection. However, the mechanism by which $CD8^+$ T cells persist within the CNS is not known. We show, for the first time, that the CXCL16/CXCR6 chemokine signaling pathway is necessary for the maintenance of T_RM T cells in the CNS after recovery from CNS West Nile Virus (WNV) infection. Using an unbiased single-cell RNA-sequencing (scRNA-seq) approach, we see that CXCL16 levels are elevated in activated microglia and CXCR6 levels are elevated in $CD8^+$ T cells in the cortex and hippocampus 25 days after WNV infection. Additionally, 52 days after viral recovery, CXCR6 KO mice have reduced numbers of T_RM $CD8^+$ and viral-specific T cells, as well as decreased microglial activation, in the cortex and hippocampus compared to WT mice. We further show that CXCR6 KO mice recover synapses in the CA3 region of the hippocampus, which are eliminated during acute infection, by 52 days after viral recovery. Here, we have identified an

underappreciated role of the CXCL16/CXCR6 pathway in the maintenance of T_RM cells in the CNS parenchyma. Targeting of this pathway may be a successful approach for the prevention of learning and memory deficits that persist after recovery from viral encephalitis.

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Poster

P320. Neuroinflammation: HIV and Infections, and Elicit Drugs

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P320.07

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

Support: NIH R01 MH087332
NIH R01 MH104131
NIH R01 MH105330
NIH R01 DA052209
NIH P50 DA026306

Title: Chronic, low dose methamphetamine reveals sexual dimorphism in memory performance impaired by exposure to HIV-1 Tat protein

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Abstract: We investigated the effects of chronic, low-dose methamphetamine (METH) use in the context of HIV infection of the brain. Therefore, we subjected transgenic mice that express a tetracycline-inducible viral protein Tat in the brain (iTat mice) at 4 months of age to a 12-week METH regimen starting week 1 at 0.5 mg/kg s.c., 1 x day, step-wise increase by 0.5 mg/kg with each injection over 5 days (Mon-Fri), followed by 11 weeks of 1 x 2.5 mg/kg/day. During week 4, the mice received Doxycyclin (Dox, 100 mg/kg, i.p.) for induction of Tat expression. The mouse cohort included rtTA-positive TRE-Tat-negative control animals, which cannot express Tat upon Dox injection, and comprised about 50 % females and males. Four months after METH exposure, at 11-12 months of age, all iTat-tg mice underwent behavioral testing, including optomotor test of vision (OPT), locomotor activity (LM), novel object recognition (NO) and Barnes maze test (BM; 4 day acquisition + probe trial). Tat compromised performance in the NO and BM paradigms but the combination of METH and Tat resulted unexpectedly in a virtually

normal performance in the probe trial of the BM. However, the NO paradigm revealed a sexual dimorphism in that METH alone only compromised males, whereas females, in contrast to males, displayed no discrimination between familiar and novel object after exposure to Tat with or without METH. Thus, METH apparently compromises recognition but not spatial memory in a sex-dependent fashion.

Disclosures: M. Kaul: None. **R. Maung:** None. **D. Ojeda-Juárez:** None. **P. Sanchez-Pavon:** None. **A.B. Sanchez:** None. **A.J. Roberts:** None. **&. TMARC Group:** None.

Poster

P320. Neuroinflammation: HIV and Infections, and Elicit Drugs

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Program #/Poster #: P320.08

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

Support: NIGMS RISE R25GM082406
RCMI MD007579

Title: Hiv-1 nef expression in astrocytes of the nucleus accumbens increases cocaine-induced seeking behavior in rats

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Abstract: Recreational use of cocaine increases the risk of acquiring HIV and exacerbates HIV-related disease by increasing replication and damage to the central nervous system (CNS). In addition to cellular injury, studies have shown that cocaine can also alter the brain's reward system and could work synergistically with viral proteins to promote neuronal injury. Production of HIV neurotoxins contributes to HAND even with cART-suppressed replication. When HIV replication is well-controlled, the neurotoxic HIV-1 Nef protein is more likely to cause pathology than other viral proteins because it is produced by astrocytes. In this study, we focus on determining the impact of HIV-1 Nef on cocaine-seeking behavior in rats. We hypothesize that Nef will increase sensitivity to cocaine leading to an enhanced drug seeking. Using Sprague Dawley (SD) rats, we infused a lentiviral vector expressing Nef from an astrocyte specific GFAP promoter into the nucleus accumbens (NAc), an important structure involved in the development of cocaine abuse. After recovery, we used an addiction paradigm where rats were exposed to 10 sessions of a short-access cocaine self-administration, followed by 14 sessions of extinction. After extinction, all rats underwent a cue-primed reinstatement followed by a session of cocaine-primed reinstatement three days later. Rats were then sacrificed for further molecular analysis by

immunofluorescence and western blot. Our preliminary data demonstrated that SD rats expressing Nef in the NAc had an increased sensitivity to cocaine during the self-administration sessions. In addition, the Nef group reinstated similarly to the control group during cue-primed reinstatement. However, during cocaine-primed reinstatement, the Nef group increased cocaine seeking compared to the control group. These data suggest that an enhanced cocaine seeking in Nef animals may be driven by a heightened sensitivity to cocaine. Moreover, future studies will focus on understanding the principal mechanisms involved in the co-exposure of HIV-1 Nef and cocaine-related extracellular glutamate regulation.

Disclosures: J. Pla-Tenorio: None. **M. Alvarado-Torres:** None. **G. Rodriguez-Torres:** None. **M. Cruz-Rentas:** None. **B. Velazquez-Perez:** None. **M. Colon-Romero:** None. **M. Sepulveda-Orengo:** None. **R.J. Noel:** None.

Poster

P320. Neuroinflammation: HIV and Infections, and Elicit Drugs

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

Support: NIH Grant R01DA050540

Title: Methamphetamine Augments HIV-1 gp120 Attenuation of Synaptic Transmission and Plasticity in Rat Hippocampal Slices

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Abstract: Methamphetamine (Meth) abuse and human immunodeficiency virus type 1(HIV-1) infection are two major public health issues worldwide. Being frequently comorbid with HIV-1 infection, Meth abuse exacerbates neurocognitive deficits in HIV-1-infected individuals even in the era of combined antiretroviral therapy. While a large body of research has studied the individual effects of Meth and HIV-1 envelope glycoprotein 120 (gp120) in the brain, far less has focused on their synergistic influence. Moreover, it is well-documented that the hippocampus is the primary site of spatial learning and long-term memory formation. Alteration of synaptic transmission and plasticity in the hippocampus is believed to impact cognitive function. To uncover the underlying mechanisms of the increased incidence and severity of HIV-1-associate neurocognitive disorders (HAND) in HIV-1-infected patients with Meth abuse, we investigated acute individual and combined effects of Meth and gp120 on synaptic transmission and plasticity in CA3 to CA1 synapses in rat hippocampus, a brain region known to be vulnerable to HIV-1 infection. Our results showed that localized application of Meth and gp120 each alone onto the

CA1 region of hippocampal slices reduced short-term dynamics of input-output responses and frequency facilitation, and attenuated long-term potentiation (LTP) induced by high frequency stimulation as well as theta burst stimulation. A synergistic augmentation on synaptic plasticity was observed when Meth and gp120 were applied in combination. Paired-pulse facilitation results exhibited an altered facilitation ratio, suggesting a presynaptic site of action. Further studies revealed an involvement of microglia NLRP3 inflammasome activation in Meth-induced augmentation of gp120-associated attenuation of LTP. Taken together, our results demonstrated Meth augments gp120-mediated attenuation of synaptic plasticity in the hippocampus. Since LTP is the accepted experimental analog of learning at the synaptic level, Meth augmentation of gp120 attenuation of LTP may underlie Meth exacerbation of HAND observed clinically.

Disclosures: Y. Zheng: None. **B.C. Reiner:** None. **J. Liu:** None. **H. Xiong:** None.

Poster

P320. Neuroinflammation: HIV and Infections, and Elicit Drugs

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

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Title: Group A streptococcus necrotizing soft tissue infections induce hippocampal inflammatory changes in HLA-II transgenic mice

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Abstract: The crosstalk between peripheral infection-induced inflammation and the central nervous system is becoming increasingly evident. *Streptococcus pyogenes* (Group A Streptococcus, GAS) bacteria cause a spectrum of human diseases ranging from self-limiting pharyngitis and mild uncomplicated skin infections (impetigo, erysipelas, cellulitis) to highly morbid and rapidly invasive life-threatening infections such as streptococcal toxic shock syndrome and necrotizing fasciitis (NF). Polymorphisms in human leukocyte antigen class-II (HLA-II) alleles are linked with differential outcomes and severity of GAS infections. While post-streptococcal neurological and neuropsychiatric conditions are associated with autoimmune

response to host mimics of GAS proteins (molecular mimicry), it is not known whether invasive GAS NF can induce neuroinflammatory changes that may lead to behavioral and cognitive dysfunction. To study the consequences of peripheral GAS skin infection on the brain, we used a NF model with and without treatment with clindamycin (CLN), the standard antibiotic care, using HLA-II transgenic mice that mimic inflammatory responses and outcomes as seen in humans. Mice expressing HLA-II DR3 or DR4 alleles were divided into three groups: uninfected controls, GAS infected, and GAS infected with resolution by CLN treatment (10mg/kg/5 days). The groups were monitored for 15 days post-infection. Skin GAS burden and hippocampal mRNA levels of inflammatory markers S100A8/A9, IL-1β and IL-33, inflammasome-related Casp1 and NLRP6 were elevated in DR3 but not DR4 mice. The levels of these markers were significantly reduced following CLN treatment in DR3 mice. Although GAS was not detectable in the brain, astrocyte and microglia activation were evident from increased GFAP and Iba-1 mRNA levels and immunoreactivity in HLA-II DR3 mice. Collectively, our data suggest a skin-brain axis during GAS NF, and the proinflammatory "cytokine storm" implicated in the pathogenesis of invasive GAS infections may regulate neuroinflammatory changes and glial phenotype alterations in an HLA-II allele-specific manner. Further investigation is warranted for developing therapeutic strategies to attenuate long-lasting brain inflammation due to latent GAS infection in those who are genetically "at-risk" and prevent possible cognitive dysfunction as a sequela.

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Poster

P339. Traumatic Brain Injury: Pre-Clinical Studies

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P339.01

Topic: C.10. Brain Injury and Trauma

Support: CNRM 65310-309318-6.01 and 65310-310185-6.01

Title: Isoflurane exposure has no effect on behavioral outcomes following repeated diffuse brain injury in male and female mice

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Abstract: Experimental brain injury is usually performed under anesthesia for humane reasons. However, questions arise regarding the clinical relevance of the employment of anesthesia, as well as concerns of neuroprotective properties of anesthetic agents such as isoflurane. The goal

of the current study was to determine the effects of isoflurane exposure on anxiety- and depressive-like behaviors following repeated diffuse brain injury in mice. Male and female mice were placed in restraint cones and randomly assigned to one of the following groups: 1) 4X (24-hr interval) traumatic brain injury (TBI; induced with the Closed-Head Impact Model of Engineered Rotational Acceleration (CHIMERA) device) with isoflurane (approximately 4 min/day), 2) 4X sham procedures with isoflurane (approximately 4 min/day), 3) 4X TBI with no isoflurane, and 4) 4X sham procedures with no isoflurane. Righting times were observed following injury procedures; almost all unanesthetized mice regained the righting reflex immediately following injury whereas mice that underwent anesthesia remained in a supine position for several minutes. For approximately seven weeks following TBI or sham procedures, mice were tested for anxiety-like behaviors in the open field test (OFT) and elevated plus maze (EPM), and for depressive-like behaviors in the three-chambered sociability test and tail suspension test (TST). The effects of TBI on anxiety-like behaviors were test and/or time-dependent; injured mice were more anxious (displayed more thigmotactic behavior) in the OFT than sham mice on Day 4 following the final injury, but spent more time in the open arms of the EPM than sham mice on Day 20 following injuries, suggesting reduced anxiety or increased risk-taking behaviors. TBI had no effect on social behaviors on Day 32 following injury, but there was an injury by sex interaction effect on immobility time in the TST on Day 40, with injured males having less immobility time than sham-treated males. Aside from time to regain the righting reflex, isoflurane exposure had no effect on any measured behaviors. Pathological analysis is on-going, but preliminary data suggest that this TBI model induces mild damage limited to white matter. Overall, these data suggest that brief durations of isoflurane exposure in a nonsurgical TBI model do not affect the functional consequences of mild, repeated TBI in mice.

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Poster

P339. Traumatic Brain Injury: Pre-Clinical Studies

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Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P339.02

Topic: C.10. Brain Injury and Trauma

Support: UCB Biopharma SPRL

Title: Early intervention with levetiracetam or brivaracetam prevent injury-induced changes in AMPA receptor expression after traumatic brain injury in rats

Authors: *D. LING[1], R. ZANCA[2], A. SANJANA[1], P. QUAN[1], A. MEJIA-BAUTISTA[1], T. RODRIGUEZ[2], J. H. GOODMAN[3], P. A. SERRANO[2];

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Abstract: One of the pathologic sequels of traumatic brain injury (TBI) is the development of epilepsy. In the controlled cortical impact (CCI) model of TBI in rats, severe cortical trauma leads to posttraumatic epileptogenesis (PTE) characterized by evoked and spontaneous ictal-like discharges. We have found that timely, post-injury administration of the pyrrolidone antiseizure drugs (ASDs) levetiracetam (LEV) or brivaracetam (BRV) can prevent PTE after CCI injury. In this study, we examined the effects of post-injury administration of BRV and LEV on injury-induced changes of glutamatergic AMPA receptor subunit expression. Rats were subjected to severe CCI trauma (2.0 mm depth) at post-natal day 25 – 30. Randomly selected CCI-injured rats were given a single dose of LEV (150 mg/kg, IP) or BRV (21 mg/kg, IP) immediately after injury, while drug-control subjects received only the saline vehicle. Sham-injured control rats were not subjected to CCI injury. Three weeks after injury, rats were euthanized and their brains snap-frozen for western blot analysis. Hemispheres ipsilateral to the CCI site were evaluated for GluA1 and GluA2 levels. We found that the ratio of GluA1 to GluA2 levels in CCI-injured rats were significantly higher than those in sham-injured subjects ($p < 0.05$), which would be consistent with an increase in synaptic excitation due to a potential shift towards calcium-permeable AMPA receptor expression. Analysis of tissue from CCI-injured rats treated with LEV or BRV suggests that early intervention with these ASDs prevented the injury-induced change in the GluA1/GluA2 ratio. These findings may provide insights to the pathogenic mechanisms of TBI that give rise to neural circuit hyperexcitation and epileptogenesis, and to the antiepileptogenic mechanisms of LEV and BRV.

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Poster

P339. Traumatic Brain Injury: Pre-Clinical Studies

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Program #/Poster #: P339.03

Topic: C.10. Brain Injury and Trauma

Support: NIHM R01 MH105623
Center for Neuroscience and Regenerative Medicine
Transforming Technology for the Warfighter Program HU00012020014 M4

Title: Increased sensitivity to ketamine’s behavioral effects in mice exposed to mild blast induced traumatic brain injury.

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[1]Pharmacol. and Mol. Therapeut., [2]Gynecologic Surgery & Obstetrics, Uniformed Services Univ., Bethesda, MD

Abstract: Neurobehavioral deficits emerge in approximately 50% of patients following a mild traumatic brain injury (TBI). These effects may persist for months following recovery from the initial trauma. Ketamine is used frequently in medicine for management of anesthesia, pain and psychiatric disorders. However, patients with a history of TBI may develop differential sensitivity to the neurological and behavioral effects of ketamine, complicated their treatment. Therefore, this series of studies examined male C57BL/6J mice exposed to a single blast overpressure, inducing a mild blast TBI (mbTBI), for indications of altered sensitivity to ketamine at varying times after injury. Dystaxia (altered gait), diminished sensorimotor gating (reduced prepulse inhibition) impaired working memory (step-down inhibitory avoidance), and behavioral performance in the forced swim test and the sucrose preference test were evaluated following intraperitoneal injections of R,S-ketamine hydrochloride in mbTBI and sham animals. Dynamic gait stability was compromised in mbTBI mice on day 7 and 35 post injury and further exacerbated following ketamine administration. On day 14 and 42 post injury, prepulse inhibition was robustly decreased by mbTBI, which ketamine further reduced. Ketamine-associated memory impairment was apparent selectively in mbTBI animals 1 and 24 h post shock (tested on day 15/16 post injury). Blast exposure increased immobility scores (day 28) and reduced sucrose consumption (Day 74), and these effects were reversed selectively by ketamine treatment. Together these data demonstrate enhanced sensitivity to ketamine over an extended period in mice following a TBI. The results endorse the potential of ketamine as a treatment for neuropsychiatric complications that emerge after TBI but urge caution in clinical practice for enhanced sensitivity to its side effects in this patient population.

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Poster

P339. Traumatic Brain Injury: Pre-Clinical Studies

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Program #/Poster #: P339.04

Topic: C.10. Brain Injury and Trauma

Support: WVU PSCoR

Title: Development of a touchscreen device for assessing pig cognition

Authors: *C. VONDER HAAR[1], K. M. PECHACEK[1], S. K. WAMPLER[1], W. AO[1], M. GRACE[1], C. L. FLOYD[2];
[1]Ohio State Univ., Columbus, OH; [2]Physical Med& Rehabili, Univ. of Utah, Salt Lake City, UT

Abstract: Porcine models of disease have become prominent for a number of disorders with relevance to cognition, including traumatic brain injury, Alzheimer's disease, Huntington's disease, and exposure to neurotoxins. Despite the importance of cognitive dysfunction for each of these cases, relatively few studies focus on assessment of behavioral outcomes, likely due to a lack of readily-available tools. To address this problem, we developed a touchscreen for assessing pig behaviors. A Raspberry Pi microcomputer was used to control a touchscreen. Custom programs were written in Python using the tkinter package for a graphical user interface. A peripheral printed circuit board provided interface with a 28-volt pellet dispenser (1 gram fruit-flavor sucrose). Two iterations of this device were tested: version 1.0 with a capacitive touchscreen and screen-adjacent pellet dispenser, and version 2.0 with a resistive touchscreen and remote pellet dispenser. All mechanical/electrical elements worked well and can be acquired or produced for a low cost. Pigs acquired a response to the touchscreen in the first session and took another 2-3 sessions to become accurate to smaller illuminated squares on the screen. Pigs then underwent testing on a delay discounting task and showed a robust aversive response to the delay. Ongoing experiments are assessing a complex discrimination task. Version 1.0 of the device worked reasonably well for training, but the capacitive touchscreen was not amenable to testing without an experimenter to wipe the screen down from saliva and ultimately failed due to a crack in the LCD display element. Version 2.0 uses a commercial-grade resistive touchscreen and testing is ongoing with this device. Ultimately, pigs were readily trained to the touchscreen and can rapidly be tested on cognitive assessments. Additional training sessions (AM/PM) may improve upon the speed of this process even further. On the physical side, some components require additional hardening against the strength of pigs and studies are ongoing to optimize the components so that the device may be brought to labs studying disorders with relevance to cognitive function.

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Poster

P339. Traumatic Brain Injury: Pre-Clinical Studies

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Program #/Poster #: P339.05

Topic: C.10. Brain Injury and Trauma

Support: NINDS Grant R01-NS110905

Title: Discrepancies in chronic behavioral deficits and inflammatory responses between male and female rats following traumatic brain injury

Authors: *S. K. WAMPLER[1], K. M. PECHACEK[1], M. A. FRANKOT[2], C. VONDER HAAR[1];
[1]The Ohio State Univ., Columbus, OH; [2]West Virginia Univ., Morgantown, WV

Abstract: Traumatic brain injury (TBI) often results in chronic deficits in decision making and impulsivity. Neuroinflammation is a potential contributor to the development of such symptoms following brain injury. Our lab previously demonstrated that brain injury increased impulsivity and decreased optimal decision making in male rats. The current study compared long-term behavioral outcomes on tasks of impulsivity (five-choice serial reaction time task) and risky decision-making (rodent gambling task) in male rats versus female rats after TBI and compared that against histological measurements of glial inflammatory activity. After training, rats were given a bilateral frontal controlled cortical impact injury. After one week of recovery, rats were retested for 11-15 weeks and then perfused and immunohistochemistry stains performed for IBA-1 and GFAP. Both male and female rats had significant and chronic increases in impulsivity and reduced optimal decision-making, however the magnitude of this effect was less pronounced in female rats. Conversely, female rats displayed significant increases in IBA-1+ microglial cells in the perilesion area, while male rats did not. Female rats also had significantly increased GFAP+ astrocytic cells. These data suggest that the inflammatory response may not be as directly tied to the development of chronic behavioral deficits or that there are critical sex differences in inflammation which converge upon similar functional outcomes. It is important to continue to investigate the effects of TBI in females to determine effective treatment strategies for females as well as males.

Disclosures: S.K. Wampler: None. **K.M. Pechacek:** None. **M.A. Frankot:** None. **C. Vonder Haar:** None.

Poster

P339. Traumatic Brain Injury: Pre-Clinical Studies

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Program #/Poster #: P339.06

Topic: C.10. Brain Injury and Trauma

Support: NINDS Grant R01-NS110905

Title: Cueing Risky Outcomes Impairs Decision-Making, Increases Impulsivity, and Augments Traumatic Brain Injury-Induced Deficits in Rats

Authors: *K. M. PECHACEK[1], C. A. FUERTES[2], S. K. WAMPLER[1], C. VONDER HAAR[1]; [1]The Ohio State Univ., Columbus, OH; [2]West Virginia Univ., Morgantown, WV

Abstract: Traumatic brain injury (TBI) is a leading cause of disability world-wide and results in the development of psychiatric symptoms including risky decision-making, impulsivity, and substance dependence. It is not well known how substance dependence develops after TBI, but dopamine dysfunction and behavioral impairments such as risky decision-making and impulsivity have important roles. Cues related to drug use can induce drug-seeking behavior and relapse, which are of relevance. The goal of this study was to assess the role of cues on decision-making and impulsivity, determine how these interact with post-TBI behaviors, and evaluate dopamine modulation via pharmacological challenges. Rats were trained on the rodent gambling task (RGT), an operant task that measures risky decision-making and impulsivity, or the cued version of this task (the cRGT). The RGT has four different choice options with varying corresponding probabilities of reinforcement or punishment, with two safe choices (lower magnitude of reinforcement, but more optimal) and two risky options (higher magnitude of reinforcement, but overall detrimental). In the cRGT, the cues increase in complexity and variability associated with the size of reinforcement and drive risky decision-making. After a behavioral baseline was established, rats received moderate-to-severe bilateral frontal controlled cortical impact injuries or sham procedures. Starting at 10 weeks post-injury, two acute dopaminergic drug challenges were given to probe potential dopamine system alterations: amphetamine (0.3, 1.0, 1.5 mg/kg) and methylphenidate (0.5, 2.0, 5.0 mg/kg). Prior to injury, cues increased impulsivity and changed the choice prolife on the RGT (*p's* < 0.001). Cues and TBI decreased optimal decision-making in an additive fashion (*p's*< 0.001). Additionally, cues increased impulsivity as an additive effect in sham but not TBI rats ($p < 0.001$; $p = 0.059$). For the dopaminergic manipulations, both amphetamine and methylphenidate did not affect optimal decision-making or impulsivity. Further research is needed to understand the biological mechanism by which cues exert their effects on decision-making and impulsivity.

Disclosures: K.M. Pechacek: None. **C.A. Fuertes:** None. **S.K. Wampler:** None. **C. Vonder Haar:** None.

Poster

P339. Traumatic Brain Injury: Pre-Clinical Studies

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Topic: C.10. Brain Injury and Trauma

Support: CONACyT T-FOSISS No. FIS/IMSS/PROT/1769

Title: Early life stress effects on cognitive performance after a mild traumatic brain injury in adult male rats

Authors: *A. SALINAS GARCÍA, J. ZAMUDIO FLORES, J. MEJÍA OLIVA, N. LAJUD;
Ctr. de Investigación Biomédica de Michoacán, Inst. Mexicano de Seguridad Social, Morelia, Mexico

Abstract: Early life stress (ELS) increases the vulnerability to a pediatric traumatic brain injury (TBI). However, weather if this effect is maintained when the injury is performed in adults remains unknown. For this reason, we evaluated if exposure to ELS in the form of maternal separation affects cognitive performance after a mild and severe TBI in adults. A controlled cortical impact injury of mild (mTBI) and moderate (TBI) intensities (2.6 and 2.8 mm respectively at 4m / s) was performed in control and maternally separated (MS, 3 hours a day from postnatal day 1 to 21) adult rats (110 days of age). Cognitive performance was evaluated in the Morris water maze (MWM) from post-injury day 14 to 18 and a probe trial was assessed on post injury day 19. As expected, our results indicate that moderate TBI but not mTBI affects cognitive performance in the learning phase of the MWM; however, MS + mTBI rats showed a significant impairment in cognitive performance when compared to Shams. No differences were observed on the probe trial. In conclusion, our data indicate that MS increases cognitive deficits after a mTBI but not after a more severe injury. This results highlight the importance the interaction between early environment and the brain insults later on life and suggest a possible difference in adaptation depending on the severity of the second hit.

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Poster

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Program #/Poster #: P339.08

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant NS110609
Research Advisory Committee, Children's Hospital of UPMC
NIH T32 NS007433-22

Title: Neurobehavioral and physiological effects of traumatic brain injury in spontaneously hypertensive rats

Authors: *R. REDDY, P. RENNERFELDT, E. H. MOSCHONAS, B. WEHRMEYER, T. RANELLONE, S. BALAJI, J. P. CHENG, A. E. KLINE, C. O. BONDI;
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Abstract: Introduction. 2.8 million people sustain a traumatic brain injury (TBI) yearly, with many experiencing long-term disabilities often exacerbated by pre-existing comorbidities. In the USA, an estimated 50% of adults suffer from hypertension, which may lead to heart attacks, reduced blood flow to muscles, strokes, and premature death. There is a critical need to investigate TBI in hypertensive rats to better characterize neurological, physiological, and cognitive impairments, and to enhance translatability. This study explores the effects of TBI on Spontaneously Hypertensive Rats (SHR) via a battery of behavioral assays, such as motor coordination/balance, hippocampal-dependent learning, sustained attention, and anxiety-like symptoms. Hypothesis: Hypertension will exacerbate TBI-induced deficits. Methods. We conducted a pathophysiological study on SHR rats compared to normotensive Wistar Kyoto (WKY) rats. Rats were randomly assigned to receive either a cortical impact injury or a sham injury. Adult male SHR-TBI rats exhibit 10% higher heart rate and 30% higher mean arterial pressure than injured WKY counterparts. TBI and sham SHR rats underwent beam-walk tests (post-operative days 1-5) and water-maze (postoperative days 14-19) testing to assess motor coordination and spatial learning, respectively. Open field testing (OFT) and the shock probe defensive burying (SPDB) task were then performed to examine anxiety-like and active/passive coping behavior, respectively. To examine sustained attention and distractibility, a 3-choice serial reaction time task (3-CSRT) was utilized in a separate cohort of SHR rats. Before surgery, rats underwent 3-CSRT training for 3-5 weeks to a 2 s cue in operant chambers. Starting on post-op day 14, rats underwent 10 days of 3-CSRT re-testing. Results. Injured SHR rats display impaired beam-walking capability, as well as reduced learning compared to SHR shams ($p<0.05$). SHR TBI rats presented more immobility and anxiety-like behavior in comparison to SHR shams, seen as reduced center area exploration in OFT and less time approaching and burying the shock probe in SPDB ($p<0.05$). SHR TBI rats also displayed markedly reduced percent accuracy and increased omissions during 3-CSRT suggesting impairments in sustained attention ($p<0.05$). Conclusions. This array of behavioral paradigms show behavioral impairments that result from TBI in SHR rats, some more pronounced than TBI- impairments previously shown in adult male Sprague-Dawley rats. Significance. Understanding the impact that underlie conditions like hypertension can have on TBI pre-clinically is critical to developing clinically-relevant therapies.

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Poster

P339. Traumatic Brain Injury: Pre-Clinical Studies

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Topic: C.10. Brain Injury and Trauma

Support: NIH Grant NS110609
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University of Pittsburgh Rehabilitation Institute

Title: Instrumental learning and attentional set-shifting during adolescence: A comparison of two models of pediatric TBI

Authors: *S. BALAJI, P. RENNERFELDT, B. WEHRMEYER, E. H. MOSCHONAS, T. RANELLONE, J. P. CHENG, N. S. RACE, A. E. KLINE, C. O. BONDI;
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Abstract: Traumatic brain injuries (TBIs) account for approximately 800,000 emergency department admissions of children in the U.S. yearly. Survivors have a higher risk of repeated mild insults or concussions, known as rmTBIs, which can lead to delayed learning and development as well as enhanced propensity for attentional and mood disorders. Preclinical models of attention after TBIs have not been cross-referenced across different injury types, leaving the possibility that injury modality differentially alters behavior. We thus aimed to compare behavioral assays of preclinical rodent models following rmTBIs (i.e., repeated concussions via closed-head injury, 1 mm depth at 4 m/s) versus a one-time controlled cortical impact (CCI) (i.e., focal injury, cortical deformation depth 2.2 mm at 4 m/s). In this study we evaluated goal-directed behavior, motivation, and attention in adolescent rats following either CCI (postnatal day, PND 17) or rmTBI (PND 17-19, 3x every 24 hrs) as pediatrics. Motivation-based behavior was measured through an instrumental learning task (ILT) requiring subjects to complete nose-pokes into illuminated holes to retrieve sucrose pellets over 12 training days during adolescence, commencing at two weeks post injury. Results were analyzed using repeated-measures ANOVAs, followed by Newman-Keuls post hoc tests. The ILT revealed no significant behavioral changes between CCI and rmTBI injuries. However, we found that adolescent rats in both TBI groups completed more total trials, made fewer task irrelevant pokes, had shorter cue-to-poke and poke-to-reward retrieval latencies compared to sham rats ($p's<0.05$), contrary to our hypothesis that TBI rats would underperform. The findings suggest divergent interpretations, such as enhanced motivation, yet reduced multi-tasking and exploratory behavior in adolescent rats after pediatric TBI. Behavioral flexibility was tested by a digging-based attentional set-shifting test (AST) with increasingly difficult contingencies for a food reward. No differences were found in trials to criterion or number of errors between CCI and rmTBI rats, or between TBI groups and Sham rats, likely due to a well-described cognitive rigidity exhibited by adolescents on AST. This study justifies evaluating CCI and rmTBI injury modalities in preclinical models for attention. Further research using clinically-relevant injury modalities to predict post-injury behavioral symptoms in survivors of childhood TBIs is therefore warranted.

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Poster

P342. Brain Injury: Cellular and Molecular Mechanisms I

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Program #/Poster #: P342.01

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R01NS096281
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Title: Nlrx1-cgas-sting-mediated neuroinflammation is triggered by mitochondrial dna and worsens outcome in a mouse model of traumatic brain injury

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Abstract: Traumatic brain injury (TBI) is a leading cause of death and disability, yet there are zero Food and Drug Administration (FDA) approved treatments. Injuries occur in two phases: primary mechanical damage due to the external force, and secondary cellular cascades such as neuroinflammation that contribute to exacerbated tissue damage. The inflammatory response to TBI is highly complex and poorly understood, but is of significant interest as a therapeutic target. Interferons, a type of inflammatory cytokine, have recently been shown to be upregulated in both mouse models of TBI and human TBI patients. The cyclic GMP-AMP Synthase-Stimulator of Interferon Genes (cGAS-STING) pathway is a key mediator of the interferon response to viral infection, but how it is activated and regulated in neurotrauma remains unclear. We provide evidence that STING is activated in a murine controlled cortical impact (CCI) model of TBI, and genetic knockout (KO) of endogenous STING or its upstream mediator, cGAS, results in reduced lesion size, cell death, and cytokine production 24 hours after injury. cGAS KO animals also show reduced motor deficits 4 days after injury, with smaller lesion size 14 days post-TBI. These data suggest that cGAS-STING activity after neurotrauma is detrimental to recovery, so we sought to clarify how it is activated in sterile injury. As a pattern recognition receptor, cGAS canonically binds cytosolic viral nucleic acids to promote STING activation. By isolating the cytosolic fraction of ipsilateral (damaged) cells from the injured cortex, we identified mitochondrial DNA (mtDNA) as being present and available for cGAS binding acutely following injury. Free nuclear DNA does not appear to be enriched in the cytosol after injury, suggesting that mtDNA is the most likely ligand for cGAS. To evaluate a possible mechanism of regulation, we investigated the effects of genetic KO of NLR family member X1 (NLRX1), a protein that has been shown to inhibit STING *in vitro*. NLRX1 KO mice showed significant increases in activated STING 3 days after injury, indicating that in the absence of NLRX1, STING is hyperactive in the injured cortex. Taken together, these data suggest that cGAS-STING signaling is aberrantly activated in brain injury by cytosolic mtDNA, and is regulated in

part by NLRX1. NLRX1-cGAS-STING signaling may therefore be a potential therapeutic target for reducing neuroinflammation after TBI.

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Poster

P342. Brain Injury: Cellular and Molecular Mechanisms I

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Program #/Poster #: P342.02

Topic: C.10. Brain Injury and Trauma

Support: RO1-NS050465
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R01-NS117148
R01-NS1168383

Title: Therapeutic potential of humanin (HN) in TBI pathology

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Abstract: Background: Traumatic brain injury reduces the threshold for neurological and psychiatric disorders, and there are no therapeutic approaches.The pathology of TBI is highly complex involving inflammation, increased calcium influx, and alterations in mitochondrial activity leading to energy dysfunction and neuronal death. Humanin (HN) is a small peptide encoded by mitochondrial DNA. We have investigated the effects of HN on JAK/STAT since this pathway is engaged on cell survival via regulation of mitochondrial functions.**Methods:** Fluid percussion injury (FPI) was performed in C57BL/6J mice (n=7) and HN (40 µg/kg, i.p.) was administered after 1 and 6 hr post-TBI. Memory function in the Barnes maze was assessed one week post-TBI. Pro-inflammatory cytokines levels were assessed (cytokine array kit, Abcam, UK) in plasma and protein levels were measured in the hippocampus using quantitative western blots.**Results:** FPI reduced memory retention ($p<0.05$) compared to sham animals, and these effects were counteracted via HN treatment. Pro-inflammatory cytokines levels were elevated in the plasma of TBI mice. Protein levels of Gp130 ($p<0.05$), p-STAT3 ($p<0.01$) were increased whereas p-CREB ($p<0.01$) levels were reduced in the hippocampus of FPI mice. Interestingly, HN treatment counteracted the effects of FPI on pro-inflammatory cytokines levels in plasma and protein expression of Gp130, p-STAT3, and p-CREB.**Conclusion:** Our results show the powerful action of HN on molecular systems underlying neuronal function and

cognition, and suggest that HN treatment has neuroprotective potential against TBI-induced cognitive dysfunction.

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Poster

P342. Brain Injury: Cellular and Molecular Mechanisms I

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Title: Sleep fragmentation enhances inflammation and compromises stress-responsive circuitry following traumatic brain injury

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Abstract: Traumatic brain injury (TBI) impairs the ability to restore homeostasis in response to stress, indicating hypothalamic-pituitary-adrenal (HPA)-axis dysfunction. Many stressors result in sleep disturbances, thus sleep fragmentation (SF) provides a common biological pathway through which to study the effects of stress after injury. We hypothesize SF stress engages the dysregulated HPA-axis after TBI and exacerbates post-injury neuroinflammation. To test this, adult male and female mice were given moderate lateral fluid percussion TBI or sham-injury and left undisturbed or exposed to daily, transient SF for 7- or 30-days post-injury (DPI). Sub-acutely after injury (7 DPI), post-injury SF increased cortical expression of interferon- and stress-associated genes characterized by inhibition of the upstream regulator NR3C1 that encodes glucocorticoid receptor. Moreover, post-injury SF uniquely increased neuronal activity in the hippocampus, a key regulator of HPA-axis reactivity. By 30 DPI, chronic post-injury SF enhanced cortical microgliosis and increased expression of pro-inflammatory glial signaling genes characterized by persistent inhibition of the NR3C1 upstream regulator. Within the

hippocampus, post-injury SF exaggerated microgliosis associated with decreased CA1 neuronal activity. Electrophysiological assessment revealed both TBI and SF induced deficits in Schaffer collateral long-term potentiation compared to controls. TBI increased neuronal activity in the bed nucleus of the stria terminalis associated with suppressed activity in the hypothalamic paraventricular nucleus with post-injury SF, indicating possible enhanced inhibitory tone of the HPA-axis with stress exposure after injury. Together these data demonstrate that post-injury SF engages stress-responsive brain regions and the dysfunctional HPA-axis to enhance neuroinflammation. Therefore, external stressors that disrupt sleep have an integral role in mediating outcome after brain injury.

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Poster

P342. Brain Injury: Cellular and Molecular Mechanisms I

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Topic: C.10. Brain Injury and Trauma

Support: NIH R21NS095158
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Title: High Frequency stimulation acutely after injury to in vitro neuronal networks is neuroprotective

Authors: C. P. ROJVIRAT[1], T. D. NGUYEN[2], ***J. R. BERLIN**[3];
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Abstract: Mild traumatic brain injury (mTBI) accounts for majority of all TBI cases. Patients can suffer acute and persistent symptoms but, despite the prevalence of significant impairments, treatment options are limited, and interventions to prevent symptomology are lacking. One impediment towards improving this situation is our lack of understanding how and why brain networks change acutely after injury. The goal of this research was to understand acute injury-induced changes in *in vitro* neuronal networks and then apply neuroprotective interventions to mitigate those changes. To capture acute changes in neuronal network dynamics, Ca^{2+} fluorescence imaging was used to record activity from large numbers of cells during spontaneous and stimulated activity in cultures of rat neocortical neurons. Stimulated activity was initiated by

single-cell photolysis of caged-glutamate (photostimulation) to reveal functional connections, or links, between neurons. Experiments were performed in the presence of bicuculline to characterize the properties of excitatory neurotransmission. To mimic the effects of mTBI, neuronal networks, grown on 0.005in thick silicone membrane, were stretched at two different strain and strain rate levels, 45%, $20s^{-1}$ or 70%, $35s^{-1}$. Compared to non-injured controls, injured neurons showed a decrease in link stability, number of neurons responsive to photostimulation, and participation of neurons in and amplitude of spontaneous activity at both strain levels. In addition, network parameters after injury were characterized by increased variability when compared to non-injured controls. Thus, network activity was acutely depressed and highly variable after injury. Cell excitability, measured as the Ca^{2+} response to direct photostimulation, also decreased after injury in a strain-dependent manner. A number of purported neuroprotective interventions were assessed after injury, but only one, brief periods of high frequency electrical stimulation (HFS: 100 Hz field stimulation, 1 s duration, repeated 3 times), was shown to prevent injury-induced changes in network parameters, including variability, and could prevent injury-induced reductions in cell excitability. Preliminary results suggest HFS-induced neuroprotection may involve activation of GluN2B receptors, an NMDA receptor sub-type known to play a critical role in Ca^{2+}-dependent neuroplasticity. These results point to future experiments that will explore the mechanism of neuroprotection by high frequency stimulation.

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Poster

P342. Brain Injury: Cellular and Molecular Mechanisms I

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Title: Injury induces lipid-droplet associated microglia with defective phagocytosis

Authors: *P. MANCHANDA[1], P. PRAKASH[1], E. ROGERS[2], Z. ZHANG[2], T. B. BEAUCLAIR[2], A. D. DUNHAM[1], R. SHI[2], G. CHOPRA[1];
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Abstract: Traumatic Brain Injuries (TBI) are correlated with an elevated risk of developing Alzheimer's Disease (AD) and related dementias, known as post-TBI AD. Microglia, the resident immune cells of the brain, are professional phagocytes that are essential for clearing amyloid-beta (Aβ) aggregates; Aβ accumulation in the brain is related to the onset of neurodegenerative disorders, such as AD and post-TBI AD. Previous studies have indicated that microglia become dysfunctional and dystrophic in neurodegenerative and aged brains. Further studies indicate that microglia in the aged brain accumulate lipid droplets (LDs) and have a proinflammatory and defective phagocytic phenotype. Our recent work showed that Aβ exposure induces LDs in microglia in age and sex-dependent manner inducing phagocytic dysfunction using the AD mouse model (5xFAD). To determine phagocytic response, we developed and extensively characterized a pH-dependent fluorogenic human Aβ1-42 reporter (AβpH) that exhibits green fluorescence upon internalization into the acidic phagosomes of cells, allowing us to image glial uptake of AβpH in real-time in live animals. We asked if the LD associated microglia is also induced as a response to injury with similar phagocytic defect towards Aβ? To this end, we used a recently developed *in vitro* "TBI on a chip" model to induce impact injury to cells in addition to simultaneous electrophysiological and morphological observations during impact. We showed that impacted mouse microglial cells treated with Aβ have lower phagocytic capacity but increased inflammatory response with elevated production of nitric oxide, 7-days post-impact. Inflammation and phagocytosis assays were done following 1, 3- or 7-days post-impact by using AβpH. Further, using an established, clinical-relevant *in-vivo* blast TBI model, we discovered that microglia isolated from blasted mice brains accumulate more LDs compared to sham, and similar to LD accumulation seen in age-matched 5xFAD mice. These LD+ blasted microglial cells also showed phagocytic defect, similar to microglia from 5xFAD mice, suggesting a novel mechanism that may lead to post-TBI AD. Uncovering the pathophysiological mechanisms leading to dysregulated microglial phagocytosis in TBI will open new avenues for potential targets and therapeutics to combat neurodegeneration. Our work is especially relevant for military applications, as there is an alarming increase in post-TBI AD and related dementia among soldiers, imposing an enormous socioeconomic burden on military families, and society.

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Poster

P342. Brain Injury: Cellular and Molecular Mechanisms I

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Topic: C.10. Brain Injury and Trauma

Support: DE-AC52-07NA27344 /21-LW-014

Title: Adapting the brain-on-a-chip system to evaluate whether reprogramming astrocytes hold the key to healing damaged neuronal networks

Authors: *D. LAM[1], C. BOGGURI[1], J. CADENA[2], A. LADD[2], A. SEBASTIAN[1], H. A. ENRIGHT[1], N. O. FISCHER[3];
[1]Physical Life Sci. Directorate, [2]Engin. Directorate, [3]Physical and Life Sci., Lawrence Livermore Natl. Lab., Livermore, CA

Abstract: Traumatic brain injury (TBI) is a global public health concern, with a high incidental rate in military personnel. To date, there are no effective treatments available for TBI. In the last 6 years, in vivo cellular reprogramming has been an attractive approach; reducing secondary injury and replacing lost neurons in mouse models of an acute brain injury (i.e., stab or stroke), Alzheimer's and Parkinson's disease. In vivo cellular reprogramming, either chemically or by adding a single gene to the targeted cell's genome, of astrocytes or microglia causes the innate cells to take on functional characteristics of the desired neuron. Advances in this technology is now selective: reprogramming reactive astrocytes or microglia into neurons, while not affecting the healthy cells has been successful. While the technology has shown to be effective in generating neurons within the damaged or diseased brain tissue, there is no information on whether these 'new' neurons can functionally integrate into existing neuronal networks (i.e., healthy, damaged or dysfunctional). Integration into existing neuronal networks is needed for healthy brain function. At Lawrence Livermore National Laboratory, we have recently developed a 3D human brain-on-a-chip (BOC) system that integrates a 3D biological system with a novel 3D multi-electrode array (3D MEA). Here, we adapt this system to contain human astrocytes to evaluate (using western blot and immunocytochemistry) the efficiency of chemically reprogramming oxygen-glucose deprivation (OGD)-induced activation of astrocytes into neurons. Using the 3D MEA, we will evaluate the ability of new neurons (from reprogrammed astrocytes) to restore neuronal networks disrupted by OGD, the secondary injury phase of TBI. This will address whether strategies that 'add' neurons to the TBI-injured brain (i.e., cellular reprogramming or stem cell transplant) are able to reverse or mitigate the effects of TBI or if additional approaches, (e.g., electrical neuromodulation) are required to support the integration of these neurons into existing networks.
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Poster

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Topic: C.10. Brain Injury and Trauma

Title: Btbi-on-a-chip: a novel, in vitro model for mechanistic investigations of blast injuries

Authors: *T. B. BEAUCLAIR[1], E. A. ROGERS[1], Z. ZHANG[2], A. THYEN[5], L. PRACHT[3], R. SHI[4];
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Abstract: Every year, over 20,000 soldiers experience traumatic brain injuries (TBI), resulting in significant loss of income and quality of life. Further, up to 95% of these highly heterogeneous injuries are caused by explosive blasts, or bTBI. Unfortunately, there are no current treatments available and the underlying mechanisms remain elusive. However, oxidative stress is one vector thought to play a role. Recently, our laboratory successfully developed a novel approach for sub-cellular investigations of concussion injuries, utilizing neuronal networks on multielectrode arrays (MEAs), capable of providing simultaneous morphological and electrophysiological observations. By combining this unique method with our established in vivo bTBI device, we introduce “bTBI-on-a-chip,” a high resolution in vitro blast injury model with recording capabilities. The blast injury device consists of a pressure chamber and chute separated by a 0.25mm thick polyethylene terephthalate membrane. Upon ignition, nitrogen gas is delivered to the pressure chamber, rupturing the membrane, and generating a shockwave directed at the culture below. Cultures consist of primary murine cortices that have been mechanically disassociated, pooled, and seeded onto MEAs, allowed to mature for 7 days, and subsequently placed into a 75x125mm plastic membrane containing 50mL of culture media, secured via custom acrylic “lock bars” 54mm below the blast injury device. Pressure levels were determined in a series of optimization experiments: a pressure transducer with and without a sample present revealed a relatively consistent blast exposure of 120kPa, or levels comparable to those experienced by animals in our previously established in vivo model. Post-exposure, samples are returned to incubation for later analysis. The blast procedure can be completed in ~4min while maintaining temperature, pH, and osmolarity. Initial experiments have established a conservation of cellular adhesion at 24hrs post-injury application, with over 95% viability, as compared to age and procedurally matched control networks. Pilot studies utilizing standard immunocytochemical methods have demonstrated significant increases in acrolein-lysine adducts (a clinically established marker of oxidative stress) at 24 hours post-blast injury when compared to procedurally and age matched control networks. Further, increased process shearing has been shown using neurofilament staining. These preliminary results illustrate the effectiveness of our system and open the door for mechanistic studies into bTBI, while offering a semi-throughput device for investigating potential pharmaceutical interventions.

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Poster

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Topic: C.10. Brain Injury and Trauma

Support: P41EB027062
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Title: Mitochondria fragmentation in microglia as a main contributor to secondary injury progression in a human 3D triculture brain model of traumatic brain injury

Authors: *V. LIAUDANSKAYA[1], Y. MILTON[1], A. SHEVZOV-ZEBRUN[1], V. K. ROSE[1], M. R. SHAPIRO[1], M. COE[1], A. BARREIRO[1], Y. ZHANG[1], A. J. SYMES[2], M. J. WHALEN[3], I. GEORGAKOUDI[1], T. J. NIELAND[1], D. L. KAPLAN[1];
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Abstract: Therapies to reduce or prevent neurological sequelae of traumatic brain injuries (TBI) do not exist, in large part because of our limited understanding of secondary injury progression. Mitochondria malfunctioning and neuroinflammation have been linked to secondary injury propagation; however, the cascade of events preceding mitochondria disbalance and cell-specific contribution remains unknown. We aimed to evaluate the effect of mitochondria dysregulation on the pathobiology of secondary damage progression post-TBI on a cell-specific level using a physiologically relevant human cell triculture 3D in vitro neural tissue model. To establish the triculture system, human stem cell-derived neurons, primary astrocytes, and a microglial cell line were seeded in silk protein porous scaffolds and embedded in collagen gel. Live staining showed that the tricultures remained viable and without necrosis for at least 12 months. At 6 weeks post-seeding, tricultures reached a mature state and were used to study traumatic injury: neurons expressed maturation markers MAP2 and Syn1 and formed Tuj1-positive dense networks; astrocytes and microglia expressed cell-specific markers ALDH1A1 and TMEM119, respectively. Injuries were inflicted on the 3D tricultures using controlled cortical impact (CCI) (moderate injury: 6m/s speed, and 0.6mm penetration). At 24 hours after CCI, injury of tricultures resulted in 80% reduction of Tuj1 positive network ($n=12$, $p<0.001$), whereas di-cultures of neurons with astrocytes or microglia showed preservation of the network (80%, $n=12$, $p<0.001$). Neuronal network degeneration in tricultures was associated with increased mitochondrial fission and pro-inflammatory cytokine production. Treatment with an anti-mitochondrial fission inhibitor P110 (1μM) slowed secondary damage progression and neuroinflammatory response at 2 ($n=9$, $p<0.001$) and 14 ($n=4$, $p<0.05$) days after injury. Naïve

neurons treated with extracellular mitochondria isolated from astrocytes, conditioned with microglial extracellular mitochondria (primed with media from injured neurons) resulted in 50% of Tuj1 positive network loss ($n=8$, $p<0.05$), induced Drp1/Fis1 mitochondria fission ($p<0.05$), and cytokines release. However, when microglia were excluded from transfer experiments, the Tuj1 neuronal network remained intact. Using a novel physiologically relevant human 3D *in vitro* triculture model of brain injury we discovered that mitochondria fragmentation in microglia is driving secondary neurodegeneration after moderate type of brain injury. We thank the NIH (P41EB027062, S10OD021624) and the DoD (HU00012020015) for support.

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Poster

P342. Brain Injury: Cellular and Molecular Mechanisms I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P342.09

Topic: C.10. Brain Injury and Trauma

Support: NASA 80NSSC21K0273
NIGMS 1P20GM134974-01A1
NIEHS R21 S031211-01A1

Title: Detection of DNA damage response via novel genetic biodosimetry sensors permits the risk assessment of space radiation-induced neurodegeneration and cognitive decline

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Abstract: There is growing concern that exposure to galactic cosmic radiation (GCR) during long-term space exploration missions to Mars or other destinations may induce or accelerate the progression of cognitive decline and neurodegeneration. The ability of the scientific community to assess these risks has been limited by the lack of an *in vivo* tool with sufficient spatiotemporal resolution and sensitivity to observe neurons after space radiation exposure. To this end, we have developed a genetically-encoded *in vivo* biodosimetry sensor, Probe with a viRal proxy for the Instability of DNA surveillance/repair in somatic brain Mosaicism (PRISM), that combines the

transduction specificity of AAV(PHP.eB) with the instability of hypermutable mononucleotide repeat regions in order to observe neuronal DNA damage response (DDR). Using both cultured HEK293 cells and retro-orbitally injected C57BL/6J mice, we have demonstrated that PRISM reports both *in vitro* and *in vivo* genomic instability inflicted by chemical radiation mimics, including a frameshift mutagen and inducers of DNA double-strand breaks. The mechanism of action of the genetic sensors was further validated using a newly developed DNA Endonuclease T7E1 assay, capillary electrophoresis, and targeted next-generation sequencing (NGS) on an Illumina MiSeq. Furthermore, *in vivo*, DDR-induced expression of these membrane-tethered fluorescent sensors is driven by the transgenic expression of Cre recombinase under cell-type-specific promoters in pyramidal (CamkIIa-Cre) or dopaminergic (TH-Cre, Drd1-Cre, and Drd2-Cre) neurons. This will allow us to identify which neuronal cell types or brain regions associated with neurodegenerative disease are more susceptible to GCR, which may guide future therapeutic measures. Utilizing these sensors, we have demonstrated the ability to visualize single neurons with varying degrees of neurodegeneration, and are developing protocols to apply deep learning methods to achieve high-resolution scans of tissue-cleared (iDISCO) whole mouse brains and trace subcellular neurodegenerative changes. Our immediate future efforts to evaluate the onset and progression of neurodegeneration following GCR exposure will utilize these transgenic Cre-expressing mice, each administered 1×10^{11} genome copies of each biodosimetry sensor via retro-orbital injection, to implement a newly-developed method of recreating the radiation environment expected on spaceflight vehicles and extraterrestrial habitats. Ultimately, we are poised to determine how the space environment - as well as variables such as age and sex - affect brain genomic instability, structure, and function.

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Poster

P342. Brain Injury: Cellular and Molecular Mechanisms I

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Program #/Poster #: P342.10

Topic: C.10. Brain Injury and Trauma

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Title: D-serine produced by reactive glia mediates synaptic damage following traumatic brain injury

Authors: *D. ARIZANOVSKA[1], S. A. TAPANES[1], O. O. FOLORUNSO[2,3], S. E. BROWN[2], J. GRACIOLLI CORDEIRO[1], D. T. BALU[2,3], D. J. LIEBL[1];
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Abstract: Traumatic brain injury (TBI) is a major worldwide health concern, affecting an estimated 69 million people each year. A primary component of all TBI pathology is diffuse synaptic damage, observed in mild to severe TBI. However, few studies have examined the mechanisms that regulate synaptic damage within the complexity of the traumatically injured brain, where extrasynaptic N-Methyl-D-aspartate receptors (NMDARs) are thought to be important contributors. We recently showed that D-serine, the primary co-agonist for synaptic NMDARs physiologically produced by neurons, is synthesized by reactive hippocampal astrocytes following controlled cortical impact (CCI) and causes impairments in synaptic plasticity and memory. Here, we identify microglia as a novel cellular source of D-serine after injury and take advantage of genetically modified male mice to determine the role of glial D-serine in causing synaptic damage. Specifically, we employed a tamoxifen-inducible strategy to genetically eliminate serine racemase (SRR; the enzyme that converts L-serine to D-serine) or Slc1a4 (a D-serine transporter) specifically in astrocytes or microglia prior to CCI injury or sham control surgery. Evaluators were blinded to genotypes and conditions and all studies were performed in technical duplicate. Using Golgi analysis (n= 4, 3segments/cell, 3 cells/animal) to quantify dendritic spine density and morphology, we show that reactive glial D-serine contributes to synaptic damage in the CA1 region of the hippocampus; this is reflected in functional deficits on a contextual fear conditioning task (n = 10-12) 7 days after injury. To complement this genetic strategy, we pharmacologically blocked glial D-serine transporters or GluN2B-containing NMDARs after CCI and analyzed synaptic loss and memory. The neuroprotective effects of both strategies demonstrate that reactive astrocytes and microglia contribute to threshold levels of D-serine that hyper-activate extrasynaptic GluN2B-containing NMDARs, which are primed for activation due to excess extracellular glutamate. Furthermore, we examined 12 human TBI patient samples using Western blot and quantitative polymerase chain reactive (qPCR) and found that contused tissues express higher levels of SRR and Slc1a4 as compared to controls. Together, this illuminates a conserved mechanism of D-serine mediated damage in mouse and human injury and suggests a critical pathological pathway that may be targeted to protect synaptic function following TBI.

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Poster

P347. Animal Models of Brain Injury: Molecular-Cellular Mechanisms and Therapeutics

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Program #/Poster #: P347.01

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R01NR013601

Title: Deficiency in the truncated BDNF receptor trkB.T1 confers neuroprotection after traumatic brain injury in male mice

Authors: *R. SHAHROR[1], J. HE[1], Z. ZHAO[1], S. G. DORSEY[2], A. I. FADEN[1], J. WU[1]; [1]Dept. of Anesthesiol. & Ctr. for Shock, Trauma and Anesthesiol. Res., Univ. of Maryland, Sch. of Med., Baltimore, MD; [2]Sch. of Nursing and Program in Neurosci., Univ. of Maryland Baltimore, Baltimore, MD

Abstract: TrkB.T1 is a truncated isoform of the BDNF receptor—tropomyosin related kinase B (trkB). It is known that trkB.T1 lacks the intracellular kinase domain of the full length receptor and is up-regulated in multiple neurological disorders. TrkB.T1 is the sole isoform of trkB receptors expressed on astrocytes. We have previously reported in mouse spinal cord injury that trkB.T1 is predominantly up-regulated in reactive astrocytes and deletion of trkB.T1 in astrocytes reduces hyperpathic responses and improves motor coordination. However, neither the cellular mechanisms nor critical role of trkB.T1 in the pathophysiology of traumatic brain injury (TBI) are studied. Here, we investigated the role of trkB.T1 in the pathobiology of TBI using a controlled cortical impact model in adult male trkB.T1-deficient (trkB.T1$^{-/-}$) mice. We observed a rapid and persistent up-regulation of trkB.T1 mRNA in the injured cortex and hippocampus after a moderate TBI. TrkB.T1 protein expression was elevated 2-3-folds at 7 days and sustained up to 6 weeks post-injury. These changes were associated with increased astrocytic GFAP expression. Flow cytometry demonstrated increased GFAP^{+} astrocytes and NOX2 expression in astrocytes from ipsilateral brain at 7 days post-injury which were significantly reduced in the absence of TrkB.T1. Furthermore, TBI in WT mice induced phosphorylation of Akt and its downstream target GSK-3β as well as ERK1/2 in both injured cortex and hippocampus at 6 weeks post-injury which were significantly enhanced in the trkB.T1$^{-/-}$ mice. In a battery of neurocognitive behavioral tests, WT/TBI mice displayed significant cognitive deficits as demonstrated by reduced time with novel object in novel object recognition test, and reduced time spent in platform quadrant in Morris water maze test. Search strategy analysis of these mice exhibited higher reliance on looping search strategies than spatial and systematic search strategies compared with WT/Sham group. In contrast, trkB.T1$^{-/-}$/TBI mice showed better performance in these tasks, indicating improved learning and memory. Assessment of depressive-like behaviors using the tail suspension and forced swim tests revealed significantly reduced immobility times in trkB.T1$^{-/-}$/TBI mice. Taken together, our data indicate an important role for trkB.T1 in regulating secondary injury-mediated functional damage post-TBI

Disclosures: R. Shahror: None. **J. He:** None. **Z. Zhao:** None. **S.G. Dorsey:** None. **A.I. Faden:** None. **J. Wu:** None.

Poster

P347. Animal Models of Brain Injury: Molecular-Cellular Mechanisms and Therapeutics

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Program #/Poster #: P347.02

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R01NS094527

Title: Impairment of autophagy in microglia/macrophage potentiates neuroinflammation after spinal cord injury

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Abstract: Autophagy is a catabolic process that degrades cytoplasmic constituents and organelles in the lysosome, thus it serves an important role in cellular homeostasis and protection against neurodegeneration. We have previously reported that defects in autophagy contribute to neuronal cell damage and are part of the secondary injury mechanism in traumatic spinal cord injury (SCI). Recent data implicate autophagy in regulation of immune and inflammatory responses, with high levels of autophagy flux associated with anti-inflammatory, and low levels with pro-inflammatory phenotypes. To determine if autophagy may be involved in modulation of neuroinflammation after SCI, we assessed the levels of autophagy in resident microglia and infiltrating macrophages following moderate contusion SCI in male mice. Flow cytometry demonstrated temporal dysregulation of autophagic function in both microglia and infiltrating myeloid from injured spinal cord at 3d post-injury which was accompanied by increased proinflammatory cytokines. Immunohistochemistry in transgenic Cx3cr1-GFP mice confirmed accumulation of autophagosomes and inhibition of autophagy flux specifically in the activated microglia/macrophages. Beclin1 (becn1) is a core subunit of the phosphatidylinositol-3 kinase (PI3K) complex and an important component in autophagosome formation. Beclin1 heterozygous ($Becn1^{+/-}$) mice displays autophagy hypomorph. Nanostring analysis with the neuroinflammation panel demonstrated increased proinflammatory genes and decreased expression of molecules related to neuroprotection in the $Becn1^{+/-}$ mice compared to their wild type (WT) littermates at 3d post-injury. These findings were further validated by real-time qPCR, in which we observed significantly higher levels of proinflammatory cytokines. Western blot analysis showed higher protein expression levels for microglia/macrophage marker Iba-1 and autophagosome marker LC3 in $Becn1^{+/-}$ mice at 1d SCI, along with increased levels of the inflammasome marker NLRP3. Assessment of functional outcome through the Basso Mouse Scale for locomotion showed significantly worse impairments for $Becn1^{+/-}$ mice after SCI compared to WT mice. Furthermore, histology analysis for spared white matter and lesion volume at 6 weeks after SCI showed worsening tissue damage in $Becn1^{+/-}$ mice. Taken together, our data indicate that inhibition of autophagy in microglia/macrophage potentiates pro-inflammatory activation that is associated with poorer functional outcome. These findings

highlight the importance of autophagy in resident immune cells of CNS and further elucidates its role in secondary injury after SCI.

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Poster

P347. Animal Models of Brain Injury: Molecular-Cellular Mechanisms and Therapeutics

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Program #/Poster #: P347.03

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant RF1NS110637
NIH Grant R01NS110635

Title: Differential changes in plasma extracellular vesicles with age and biological sex after spinal cord injury

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Abstract: Spinal cord injury (SCI) leads to secondary brain neuroinflammation and neurodegeneration that increases dementia risk. Preclinical studies support that cognitive behavioral deficits are exacerbated with age and worse in males. However, the mechanisms contributing to SCI-induced brain dysfunction are not well understood. Cell-to-cell signaling through extracellular vesicles (EVs) has emerged as a critical mediator of neuroinflammation, including at a distance through circulation. We have previously shown that SCI in young adult (YA, 3-month-old) male mice leads to robust changes in plasma EV count and microRNA (miR) content. Here, our goal was to investigate the impact of age, biological sex, and aging on EVs after SCI. YA or aged (18-month-old) mice received T10 midline moderate contusion injury. Total plasma EVs were isolated by ultracentrifugation. Particle count and size distribution were assessed by nanoparticle tracking analysis, while individual EV protein expression was quantified by Western blot. YA mice were subjected to SCI followed by 19 months post-injury. There was no difference in particle count or size in either sex of both sham and SCI groups. Interestingly, males had increased expression of tetraspanin EV markers CD63 and CD81 after SCI, suggesting a sex-dependent difference in circulating EV subpopulations. We then evaluated plasma EVs in aged male animals at early and late timepoints following SCI. In shams, plasma EVs from aged mice had decreased count and a smaller peak size relative to YA. At 2 months post-injury, there was no significant difference in particle count or size distribution between YA and aged mice. However, aged animals showed increased expression of CD63 and CD81 with

SCI. To identify changes in plasma EV miR cargo, we performed the Fireplex® assay in YA and aged mice at 1d post-injury. While miR-145-5p, miR-206, miR-214-3p, and miR-23a-3p increased with injury in both groups, miR expression did not significantly differ alone with age or within a specific injury group. Using the same assay, we found that SCI increased miR-451a, miR-486-5p, miR-15b-5p and decreased miR-206 expression in EVs derived from the injury site in both groups. Interestingly, miR-206 decreased in EVs at the injury site and increased in plasma EVs, suggesting that this miR is shuttled through EVs into circulation. miR-206 is highly expressed in astrocyte EVs, and increased plasma miR-206 is correlated clinically with transition from mild cognitive impairment to dementia. These studies are the first to describe changes in circulating EVs after chronic SCI and in aged animals and support a potential EV-mediated mechanism for SCI-induced brain changes.

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Poster

P347. Animal Models of Brain Injury: Molecular-Cellular Mechanisms and Therapeutics

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Program #/Poster #: P347.04

Topic: C.10. Brain Injury and Trauma

Support: NIH K99NS116032 (RR)
NIH RF1NS110637 (JW)

Title: Age-related dysregulation of autophagy contributes to aggravated microglial dysfunction and long-term neurological outcome after traumatic brain injury

Authors: *R. RITZEL, Y. LI, J. HE, J. CARTER, N. KHAN, A. I. FADEN, J. WU;
Dept. of Anesthesiol. & Ctr. for Shock, Trauma and Anesthesiol. Resea, Univ. of Maryland, Sch. of Med., Baltimore, MD

Abstract: Elderly patients with traumatic brain injury (TBI) have higher mortality and poorer long-term outlook compared to younger individuals. This may contribute to the assumption that aggressive management of geriatric TBI is futile. The present study examined the long-term recovery potential and underlying mechanisms associated with advanced age in male C57BL/6 mice using a controlled cortical impact model. 18-month-old aged mice had a higher mortality rate compared to young (10-weeks-old) mice at 12 weeks post-injury. While aging alone had a significant impact on behavioral ability, the recovery slope in some, but not all, neurobehavioral tests was relatively similar between young and old mice. By 12 weeks, however, aged mice displayed significant cognitive deficits and depression. Histopathology revealed larger lesion volumes and increased microglia activation in aged TBI mice. Nanostring analysis identified

several age- and injury-specific genes that were differentially expressed in the injured brain, including those involved with the complement, phagocytosis, and autophagy pathways. Flow cytometry demonstrated temporal dysregulation of autophagic function in microglia from injured mice which was exacerbated with age and accompanied by increased neuroinflammation. Given the critical role for autophagy in promoting the cellular degradation of lipids, proteins, and damaged organelles we reasoned that a treatment employing an autophagic inducer such as trehalose may be a viable therapeutic strategy for old mice. Trehalose was administered in the drinking water (3%) starting at d1 post-injury up to 8 weeks. Aged male mice were randomly assigned to one of four groups based on surgery (sham or TBI) and treatment (trehalose or sucrose control). Behavioral testing was performed by an investigator blinded to each group. Old TBI mice treated with trehalose exhibited either delayed deficits or enhanced recovery in cognitive and motor tasks. Trehalose treatment modified expression of autophagy markers and reprogrammed the microglial response to chronic TBI. Taken together, our data indicate that microglia undergo chronic changes in autophagic regulation with both normal aging and TBI that is associated with poorer functional outcome. Boosting autophagy may be a promising therapeutic strategy for older TBI patients.

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Poster

P347. Animal Models of Brain Injury: Molecular-Cellular Mechanisms and Therapeutics

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P347.05

Topic: C.10. Brain Injury and Trauma

Title: Exploring the effects of unpredictable chronic mild stress on traumatic brain injury outcomes in drosophila melanogaster

Authors: *B. E. TOLAN, R. E. HARTMAN;
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Abstract: Traumatic brain injury (TBI) is a leading cause of neurological deficits and mortality worldwide. The consequences of TBIs include sensorimotor, cognitive, and psychological deficits, all of which may be exacerbated by chronic stress from environmental stressors. Environmental stress and TBI are both associated with inflammation, and each has the potential to induce depression and/or impair performance on cognitive and sensorimotor tasks. However, the nature of the interaction between these two nervous system insults is currently unclear. Furthermore, there are currently no proven pharmaceutical strategies to either protect against or repair nervous system dysfunction initiated by chronic stress and/or TBI. A cost efficient and widely available solution may be found in dietary sources such as pomegranate juice. Abundant

in phytochemicals, polyphenols are the most common and considered the most bioactive. These compounds have anti-inflammatory and antioxidant properties. Pomegranate juice contains ellagitannin and punicalagin, which is hydrolyzed into ellagic acid (EA). Here we show that a high-throughput *Drosophila* model of TBI induced behavioral deficits and increased mortality. Using a well-characterized high-impact trauma device, TBIs were administered to young adult (7 day old) wild-type *Drosophila melanogaster* (*N* = 1600). Following the TBI, the flies were transferred to individually housed treatment vials of 10% pomegranate juice, 10% ellagic acid, 10% sucrose, 10% ellagic acid + sucrose, or standard fly media. At 24 hours and 9 days, flies were assessed for locomotor and behavioral deficits using Rapid Iterative Negative Geotaxis (RING) and Drosophila Activity Monitor (DAM). Lifespan was also assessed. Additionally, an Unpredictable Chronic Mild Stress (UCMS) protocol, in which the flies were exposed to mild stressors (cold temperature, housing vial agitation, and light:dark cycle disruption) presented in a semi-random order over the course of a week, was associated with decreased climbing performance and higher mortality. Interestingly, flies administered both TBIs and chronic stress demonstrated increased climbing performance compared to flies only administered TBIs. Finally, dietary supplementation with pomegranate juice and/or ellagic acid resulted in increased climbing performance in flies compared to a standard diet. These results suggest that while TBI and chronic stress may result in behavioral deficits such as decreased climbing performance as well as decreased lifespan, dietary supplementation with pomegranate polyphenol, particularly ellagic acid may reduce these negative effects.

Disclosures: B.E. Tolan: None. **R.E. Hartman:** None.

Poster

P347. Animal Models of Brain Injury: Molecular-Cellular Mechanisms and Therapeutics

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Program #/Poster #: P347.06

Topic: C.10. Brain Injury and Trauma

Title: Hormetic effect of ellagic acid on longevity after traumatic brain injury

Authors: *M. M. MENDOZA[1], E. Y. CHU[2], R. E. HARTMAN[1];
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Abstract: Polyphenols (e.g., ellagic acid) are produced by plants to protect against environmental insults such as insects / animals, ultraviolet light, extreme temperatures, etc. Despite a wealth of data demonstrating that dietary consumption of polyphenols generally confers health benefits, polyphenols often have a bitter taste and mild cytotoxic properties. Therefore, hormesis, in which mild systemic stressors can activate endogenous anti-oxidant and anti-inflammatory pathways, may explain at least some of their beneficial effects. However, this

would imply that a high enough dose will ultimately become detrimental. Although our group has demonstrated neuroprotective effects of ellagic acid and related compounds in flies, mice, and humans, we have never systematically determined minimal, optimal, and maximum dosages. Here we show in a high-throughput *Drosophila* model of traumatic brain injury (TBI) that high doses of ellagic acid may indeed have negative consequences, and that these effects manifest in a sex- and injury-dependent fashion. High doses in females, for example, reduced lifespan but did not further exacerbate the reduction in lifespan associated with a blunt-force traumatic injury. High doses in males, however, had little effect on control fly lifespan, but significantly exacerbated the negative effects of injury on lifespan. These results suggest that the often-reported benefits of consuming dietary polyphenols may be at least partially explained by hormetic processes. If so, these data further accentuate the importance of determining optimal dose in all future experimental explorations of polyphenol efficacy. “More is better” may not be the best approach to dietary supplementation. These results also suggest that other sources of environmental stress may interact with polyphenol consumption. Future studies are planned to determine the biochemical pathways activated and/or inhibited at low and high doses.

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Poster

P347. Animal Models of Brain Injury: Molecular-Cellular Mechanisms and Therapeutics

Location: Virtual Only

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Program #/Poster #: P347.07

Topic: C.10. Brain Injury and Trauma

Title: Assessing the Efficacy of a Dietary Polyphenol Treatment on Space-Like Radiation Exposure in a Fruit Fly Model of Traumatic Brain Injury

Authors: *T. TOGASHI[1], A. D. TROFIMOVA[2], W. HARDEMAN[3], V. WATTS[3], G. A. NELSON[3], R. E. HARTMAN[4];
[1]Loma Linda Univ., Redlands, CA; [2]Psychology, [4]Psychol, [3]Loma Linda Univ., Loma Linda, CA

Abstract: PhD/ Principal Investigator: Richard E. Hartman/ Co-Investigator: Tori Togashi
Astronauts traveling outside the Earth’s magnetosphere on deep-space missions will be exposed to proton irradiation from the sun and galactic cosmic rays. Additionally, the tight quarters, low gravity, demanding work environment, and lack of comprehensive emergency medical care will increase both the risks and consequences of sustaining a traumatic brain injury (TBI) during the mission. Proton irradiation and TBI each have the potential to impair performance on cognitive and sensorimotor tasks, but the nature of the interaction between these two nervous system insults is currently unclear. Furthermore, there are currently no proven pharmaceutical strategies to either protect against or repair nervous system damage initiated by irradiation and/or TBI. Here we show that a high-throughput *Drosophila* model of TBI induced climbing deficits,

hypoactivity, and reduced lifespan. Exposure to low doses of proton irradiation did not have significant effects on these parameters, but patterns of behavioral deficits after radiation exposure regardless of injury status were apparent. Additionally, dietary supplementation with ellagic acid (a polyphenol found in pomegranates) significantly increased lifespan regardless of TBI or irradiation status.
These results suggest that exposure to even low levels of proton irradiation during deep-space missions (e.g., to Mars) may exacerbate the effects of nervous system insults such as TBI. We have previously shown that dietary polyphenols can increase the nervous system's resilience to irradiation (in mice) and injury (in mice and humans), and the current data demonstrate a significant increase in longevity. Thus, prophylactically supplementing the astronauts' diet with polyphenols may help to ameliorate some of the negative effects associated with their increased risks of TBI and exposure to proton irradiation.
More importantly, these findings generally demonstrate that this high-throughput fruit fly model of TBI, in combination with exposure to varying degrees of proton radiation, can be a useful, highly replicable, and inexpensive platform with which to study the viability of multiple strategies for attenuating the effects of nervous system injury.

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Poster

P347. Animal Models of Brain Injury: Molecular-Cellular Mechanisms and Therapeutics

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Program #/Poster #: P347.08

Topic: C.10. Brain Injury and Trauma

Support: Boehringer Ingelheim Veterinary Scholars Program
MWU Kenneth Suarez 2021 Summer Fellowship

Title: Assessing mild TBI-induced axonal damage in aged Drosophila melanogaster

Authors: J. M. SPIKER[1], M. VOSS[2], T. JONES[3], A. N. JUBA[4], T. C. THOMAS[5], *L. M. BUHLMAN[4];
[1]Col. of Vet. Med., [2]Arizona Col. of Osteo. Med., [3]Anat., [4]Midwestern Univ., Glendale, AZ; [5]Dept. of Child Hlth., Univ. of Arizona Col. of Med. Phoenix, Phoenix, AZ

Abstract: Traumatic brain injury (TBI) is a major contributor to neurological deficits and mortality. After the initial injury, secondary immune signaling pathway activities lead to chronic neuroinflammation and significant neurodegeneration. Low levels of chronic inflammation are observed in the aged population and may exacerbate TBI outcomes. Because *Drosophila* lack a conserved adaptive immune system, genetically modified flies uniquely facilitate isolated

observation of innate immune system activity following TBI. Release of damage associated molecular patterns (DAMPs) from degenerating axons can activate innate immune receptor signaling, triggering inflammation after mammalian TBI. To address whether DAMPs trigger the immune response that follows TBI in *Drosophila*, young and aged flies expressing membrane-bound green fluorescent protein in dopaminergic projection neurons were injured using a modified High Impact Trauma (HIT) device (Katzenberger, et al., 2013). We hypothesized that axonal degeneration will occur following TBI and that advanced age will exacerbate degeneration in this population of neurons, which are vulnerable to oxidative stress. Young and aged injured flies exhibited temporary incapacitation and abnormal wing positioning. After injury, flies were placed in an activity monitor to measure climbing, or their brains were dissected at 60m, 24h, and 7d post-injury. Interestingly, we found that climbing behavior was only decreased in injured young flies. Z-stacks of dissected brains were captured, and axon length and surface area were measured. This preliminary study provides a foundation for high throughput studies aimed at elucidating the contribution of the innate immune system to neurodegeneration after TBI.

Disclosures: J.M. Spiker: None. **M. Voss:** None. **T. Jones:** None. **A.N. Juba:** None. **T.C. Thomas:** None. **L.M. Buhlman:** None.

Poster

P347. Animal Models of Brain Injury: Molecular-Cellular Mechanisms and Therapeutics

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P347.09

Topic: C.10. Brain Injury and Trauma

Support: MWU Ken Suarez 2021 Summer Fellowship
MWU Biomedical Sciences Program

Title: Nitric oxide synthase levels transiently decrease after TBI in aged Drosophila

Authors: M. L. MCDOLE[1], T. M. MACKEY[2], L. M. BUHLMAN[2], A. N. JUBA[3], T. C. THOMAS[5], M. J. CASTRO[4], *T. B. JONES[3];
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Abstract: The degree to which secondary injury contributes to neurodegeneration after traumatic brain injury (TBI) is becoming more apparent, and aged populations have poorer outcomes. Immune signaling can contribute to secondary damage by inducing chronic upregulation of proinflammatory mediators and oxidative stress. Nitric oxide synthase (NOS), a key inflammatory mediator, is upregulated after human TBI and may contribute to disease progression by catalyzing production of peroxynitrite, a potent reactive oxygen species.

Mammals express inducible, neuronal, and endothelial NOS isoforms, which participate in processes that include inflammation, neurotransmission, and platelet aggregation. The *Drosophila melanogaster NOS* ortholog *dNOS* is expressed throughout development and adulthood and is thought to perform the functions of all three mammalian isoforms. We hypothesized that injured flies would have increased dNOS and climbing ability would decrease. Using flies harboring a GFP reporter under the control of *dNOS* expression, we measured relative dNOS levels and motor function immediately and chronically following TBI using a modified high impact trauma (HIT) device in young and aged flies (Katzenberger *et al.*, 2013). *Drosophila* brains were dissected or climbing behavior was assessed in injured *NOS-GAL4>UA2xEGFP* flies at 30 minutes, 1 hour, 24 hours, or 7 days post-injury. Brains were fixed, z-stacks captured, and GFP intensity and area of expression were analyzed. Number of climbing attempts was transiently decreased in injured young flies, while average height climbed was transiently decrease in aged flies. Interestingly, NOS expression was decreased in injured aged flies at 30 minutes post injury and recovered by day 7. These results suggest that our injury protocol did not induce an inflammatory response or that dNOS does not play a role in inflammation in adult *Drosophila*. Observations of decreased dNOS expression may reflect changes in pathways involved in neurotransmission or synapse formation. Future studies will evaluate whether dNOS levels increase after a more severe injury and whether increases correlate with more severe climbing deficits and brain nitrosylation.

Disclosures: M.L. McDole: None. **T.M. Mackey:** None. **L.M. Buhlman:** None. **A.N. Juba:** None. **T.C. Thomas:** None. **M.J. Castro:** None. **T.B. Jones:** None.

Poster

P347. Animal Models of Brain Injury: Molecular-Cellular Mechanisms and Therapeutics

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P347.10

Topic: C.10. Brain Injury and Trauma

Support: CT IBaCS Seed Grant 40

Title: Effectiveness of Beta-Hydroxybutyrate Isomers at Ameliorate Symptoms Following Traumatic Brain Injury in a Drosophila melanogaster model

Authors: *K. MOONEY[1,4], D. C. LEE[2], G. R. TANNER[3,4], J. BALSBAUGH[3], H. VAN PELT[3], K. GAVILANES[3], Y. THELUSMA[3], J. MOONEY[3];
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Abstract: Traumatic Brain Injury (TBI) events—which are increasing in both concern and incidence in contact sports—are defined as brain injuries sustained from a blow or force to the

head that disrupts normal brain function. Following TBI, individuals may experience headaches, confusion and disorientation, and difficulty speaking. Multiple TBI events may lead to Chronic Traumatic Encephalopathy (CTE), a progressive neurodegenerative condition characterized by impaired judgment and learning and memory, and personality changes including anxiety, depression, and hyper-aggressive behavior. Progressively more young athletes in contact sports are experiencing repeated TBI, making research into TBI and CTE critical. The ketogenic diet (KD) is a low-carbohydrate, adequate-protein, and high-fat diet that has been used effectively in the clinic to treat neurological disorders such as intractable pediatric epilepsy. We have shown that directly supplementing standard high-carbohydrate *Drosophila melanogaster* diets with ketone bodies (KBs; specifically, beta-hydroxybutyrate, BHB) increases lifespan and decreases male-on-male aggression following TBI events. Our results have suggested that different enantiomeric forms of exogenous BHB may vary in their effectiveness at reducing aggression and increasing lifespan. Compared with a control diet (CD) alone, dietary supplementation with either pure R-BHB (the main endogenous form), or a BHB racemic mixture, both significantly decrease number of post-TBI aggressive events in male-male pairs; racemic and pure-S-BHB lengthen latency to the first aggressive event.The mechanism by which BHB supplementation ameliorates elevated aggression and increases lifespan following TBI is poorly understood. BHB's primary metabolic function is mitochondrial ATP production under conditions of low circulating glucose levels leading to elevated brain-wide ATP/ADP ratios. However, BHB can also act as a GPCR ligand, a gene-expression regulator (via histone deacetylase inhibition), and a sirtuin protein activator (via increased cytoplasmic [NAD+]). Sir2 activation promotes cell survival through a variety of mechanisms, including activation of autophagy proteins (e.g. autophagy-related protein 8a; Atg8a) which can mediate clearance of damaged cellular components. We hypothesize that BHB works to decrease neuronal cell death following TBI by improving neuronal metabolic stability and by activating and upregulating levels of Sir2 and Atg8a. Future experiments will include confirming reduced neuronal cell death and determining NAD+/NADH ratios in whole brains from TBI-subjected flies receiving exogenous BHB.

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Poster

P352. Biomarkers of Disease and Injury: Human Studies

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P352.01

Topic: C.10. Brain Injury and Trauma

Support: Indiana Spinal Cord and Brain Injury Research Fund (43545)
Zeiss Equipment Grant

Title: Pupillary Light Reflex changes show promise as an objective biomarker in the acute phase of concussion

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Abstract: Purpose: Concussion awareness is improving, and eye care providers are increasingly involved in concussion management. Photophobia is a common symptom of concussion, yet no objective visual biomarkers of concussion currently exist. Pupillary light reflex (PLR) dysfunction has been demonstrated in chronic traumatic brain injury and is increasingly used to detect neurological dysfunction. However, to date, no studies have published results on the potential of PLR as an objective biomarker for acute concussions. In this matched cohort pilot study, we analyzed pupillary light reflexes across three stimulus intensities and investigated whether PLR changes are detectable in the acute and/or recovery phase of concussion.
Methods: Acute concussion (≤ 72 hrs) and recovery (2 wks, 8 wks, 6-18 months) data was collected on a total of n = 39 Division I NCAA collegiate athletes n = 19 concussed and n = 20 age, sex, and sport-matched controls. At each visit automated PLR measurements were collected with an SR Eyelink 1000 eye tracker and custom software. Descriptive statistics including mean, variance, and effect size with Cohen's d were performed. Finally, an ANOVA was conducted to determine the effect of injury group and stimulus intensities on outcomes.
Results: Concussed athletes consistently demonstrated diminished PLR function relative to controls. There was an effect of stimulus intensity for three of the six PLR variables. The ANOVAs revealed significant effects of concussion injury for three variables: peak constriction diameter (davg=0.57, p=0.003), peak constriction velocity (davg=0.36, p=0.041), and peak constriction latency (e.g., time to peak constriction) (davg=0.81, p=0.000).
Conclusion: Automated pupillometry evaluating the PLR shows promise as an objective biomarker for assessing acute concussion status in collegiate athletes. Consistent with existing literature, maximum constriction velocity, constriction amplitude, and constriction latency were depressed in concussed athletes relative to their matched, healthy, teammates. These findings highlight the pathophysiological relationship between vision and concussion. Further study is warranted investigating the potential use of automated pupillometry for acute concussion assessment and management.

Disclosures: T.R. Zuidema: None. **L.M. Ferris:** None. **N.L. Port:** None.

Poster

P352. Biomarkers of Disease and Injury: Human Studies

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P352.02

Topic: C.10. Brain Injury and Trauma

Support: NIH NINDS U54 NS100064

Title: Characterizing phenotypic variability in the epilepsy bioinformatics study for antiepileptogenic therapy (EpiBioS4Rx): establishing ground-truths for automated lesion segmentation

Authors: *R. GARNER[1], A. SHARMA[1], M. LA ROCCA[1], G. BARISANO[1], R. CUA[2], A. BENNETT[1], N. NOUAILII[1], E. VESIALOU[1], A. KHAN[1], A. W. TOGA[1], P. VESPA[3], D. DUNCAN[1];
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Abstract: The Epilepsy Bioinformatics Study for Antiepileptogenic Therapy (EpiBioS4Rx) is an ongoing, international, multicenter Center without Walls investigating translational biomarkers of epileptogenesis in traumatic brain injury (TBI) patients and a preclinical model of induced TBI. Prior literature has shown that injury characteristics such as lesion type, location, and extent are correlated with increased risk of posttraumatic epilepsy (PTE). In a recent study (Tubi et al. 2018), temporal lobe trauma was found to be associated with higher rates of early seizures and subsequent PTE. Therefore, assessment and evaluation of lesion phenotypes at the time of injury is critical to facilitate optimal patient care and prognosis. However, there is no automated lesion segmentation toolbox optimized for TBI. The goal of this work is to generate gold-standard lesion segmentations that will be used as ground-truths for a machine learning-based lesion segmentation software. EpiBioS4Rx has enrolled 218 patients with moderate-to-severe TBI at 14 hospitals in the US, Australia, and Russia under local IRB approval. Enrollment criteria include frontal and/or temporal hemorrhagic contusion and Glasgow Coma Scale score between 3-13 without continuous sedation at the time of enrollment. Multimodal magnetic resonance imaging (MRI) is acquired at day 14 +/- 4 days after injury. Hemorrhagic contusions and surrounding edematous tissue were labeled on T2-weighted-Fluid-Attenuated Inversion Recovery (T2-FLAIR) MRI using ITK-SNAP. At the time of submission, segmentations for 67 patients had been reviewed by neuroradiologic experts. Binarized segmentation masks were coregistered with T2-FLAIR images into MNI space and visualized using MRICron. Voxelwise overlap of registered lesion masks for 67 patients is shown in **Fig.1**. Maximum overlap in a given voxel was 22, indicating that the regions most common (e.g. ventromedial prefrontal cortex, rostral middle temporal cortex, rostral superior temporal cortex) amongst the heterogeneous cohort were involved in 32.8% of patient injuries. Existing lesion segmentation toolboxes are optimized for white matter hyperintensities (e.g. multiple sclerosis lesions) and tumors. Brain deformations caused by trauma pose difficulties for existing registration and tissue classification algorithms that cause these methods to fail on TBI cases. This preliminary work provides data to fill the critical need for validated lesion masks that can be used to train machine-learning systems for automated lesion classification. Automated Injury phenotyping will ultimately expedite injury-related biomarker identification for PTE.

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Poster

P352. Biomarkers of Disease and Injury: Human Studies

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P352.03

Topic: C.10. Brain Injury and Trauma

Support: R01 NS106957-04
R01 NS106957

Title: Cortical and Subcortical MRI Gray Matter Abnormalities Associated with Depth Electrode-Recorded Ictal EEG Onset Patterns in Human Temporal Lobe Epilepsy

Authors: *C. OWENS-WALTON[1], H. ZHENG[1], S. I. THOMOPOLOUS[1], I. FRIED[2], N. SALAMON[3], J. P. ENGEL, Jr.[4], P. M. THOMPSON[1], R. STABA[4];
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Abstract: Introduction: Direct brain recordings and experimental models of temporal lobe epilepsy (TLE) indicate that different ictal EEG seizure onset patterns are generated by different neuronal mechanisms; however, the structural abnormalities associated with these seizures are unclear. Here we sought to determine whether different seizure onset patterns are associated with unique or shared spatial distributions of MRI gray matter abnormalities.
Methods: Subjects in this research included patients with medication-resistant, focal temporal lobe seizures who had invasive EEG tests as part of their presurgical diagnostic evaluation. Depth ictal EEGs were reviewed, and patients grouped according to their predominant pattern as follows: hypersynchronous (HYP; n=17), low voltage fast (LVF; n=57), and rhythmic spiking (RS; n=29) patterns. We evaluated regional cortical thickness and subcortical volumes between patients and age- and sex-matched control groups (n=114) using mass univariate general linear models, with a Benjamini-Hochberg false discovery rate (FDR) correction used to adjust *p*-values, and a significance threshold of *p*-FDR of < 0.05.
Results: HYP participants displayed reduced bilateral entorhinal, bilateral fusiform, left postcentral, left precentral, left supramarginal, right inferior temporal, right lingual and right paracentral cortical thicknesses compared to Controls. LVF participants displayed reduced left entorhinal, paracentral, bilateral precentral and left temporal pole cortical thicknesses compared to Controls. These subjects also had greater left *pars triangularis*, right supramarginal, left transverse temporal and right rostral middle frontal cortical thicknesses compared to Controls. RS participants displayed reduced thickness of the left precentral cortex compared to Controls.

HYP participants displayed no significant differences in subcortical volumes compared to Controls. By contrast, LVF and RS groups had reduced left thalamus and putamen (LVF only), right amygdala and nucleus accumbens, and bilateral hippocampus volumes compared to Controls.
Conclusions: In complicated cases of TLE, MRI abnormalities to subcortical structures areas may indicate a more extensive, structural network supporting the onset and spread of LVF and RS seizures than HYP seizures. Results are consistent with the hypothesis that thalamus and mesial temporal limbic structures are important hubs in TLE, representing an anatomical link between temporal and extra-temporal cortical nodes of the network generating these seizures.

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Poster

P352. Biomarkers of Disease and Injury: Human Studies

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P352.04

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R01-EY028125, P41-EB017183
BrightFocus Foundation G2016030, G2021001F
Unrestricted Grant from Research to Prevent Blindness to NYU Langone Health Department of Ophthalmology

Title: Diffusion Kurtosis Imaging of Optic Radiation Damage in Glaucoma

Authors: C. PARRA[1], J. BANG[1], R. W. CHAN[1], J. M. AU[1], E. FIEREMANS[2], G. WOLLSTEIN[1], J. S. SCHUMAN[1], *K. C. CHAN[1];
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Abstract: Glaucoma is a neurodegenerative disease and is the leading cause of irreversible blindness worldwide. Currently, intraocular pressure is the only clinically modifiable risk factor for glaucoma. Characterization of other factors such as brain involvements may allow improved treatment options including neurotherapeutics. In this study, 8 early glaucoma (66.1±7.7 years old; 5 males), 19 advanced glaucoma (65.3±7.9 years old; 10 males), and 9 healthy control subjects (63.1.1±8.1 years old; 7 males) underwent diffusion-weighted magnetic resonance

imaging (DWI) at 3 Tesla using diffusion-weighting factors (b values) of 250, 1000, and 2000 s/mm^2 at 4, 20, and 60 diffusion directions, respectively covering the whole brain. Parametric maps of the conventional diffusion tensor imaging (DTI), the high-order diffusion kurtosis imaging (DKI), and the DKI-extended white matter tract integrity (WMTI) model estimates were computed from DWI using open-source DESIGNER software. These maps were then compared between subject groups via semi-automatic Tract-Based Spatial Statistics and regions-of-interest analyses of the optic radiation using ANOVA followed by post-hoc Tukey tests and trend analyses (Fig. 1). DTI revealed fractional anisotropy (FA) reduction in the optic radiation bilaterally in glaucoma. For DKI and WMTI, radial kurtosis (RK) and axonal water fraction (AWF) were reduced in the right optic radiation of advanced glaucoma to similar spatial extents, whereas intra-axonal space diffusivity (IAS_Da) showed a more localized reduction in the right optic radiation. Our findings suggest that DTI, DKI, and WMTI estimates may be feasible biomarkers to quantify and better understand trans-neuronal visual system damages in glaucoma. While FA in conventional DTI appears more sensitive to glaucomatous brain damages bilaterally, RK in DKI and AWF or IAS_Da in WMTI may reflect more specific characterizations of local white matter changes with relevance to glial and axonal integrity, respectively.

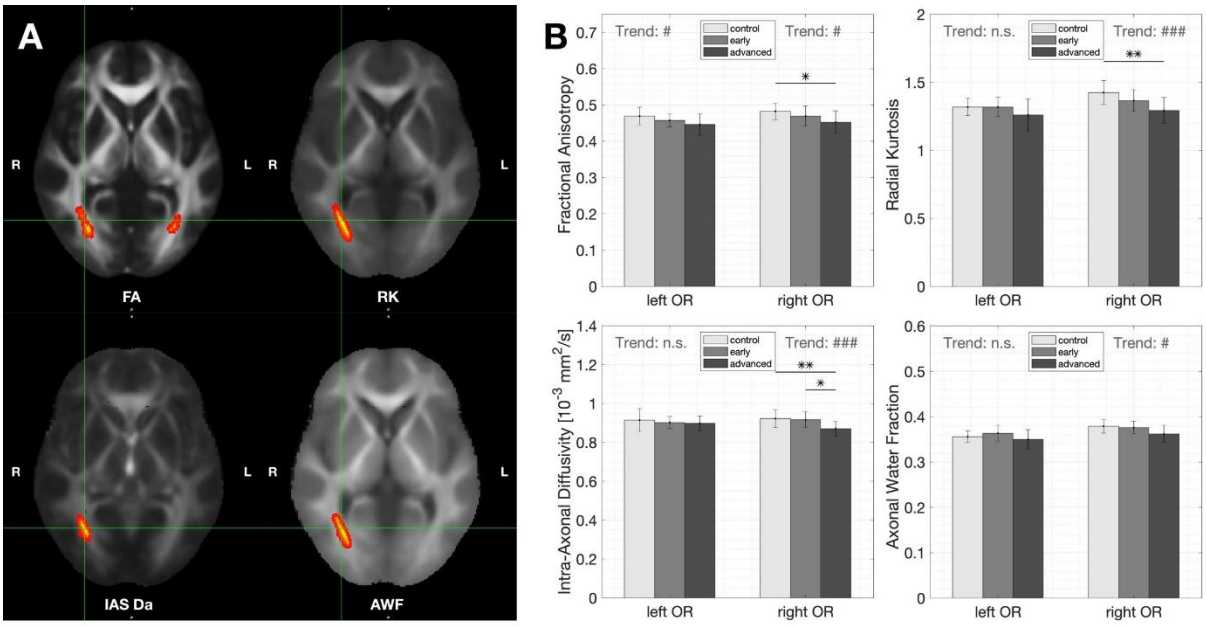


Figure 1: Tract-Based Spatial Statistics (TBSS) and Region-of-Interest (ROI) Statistical Analyses for the Optic Radiation (OR). **(A)** TBSS analysis for diffusion parametric maps including fractional anisotropy (FA) [derived from diffusion tensor imaging (DTI)], radial kurtosis (RK) [derived from diffusion kurtosis imaging (DKI)], and intra-axonal space diffusivity (IAS_Da) and axonal water fraction (AWF) [estimated from extended kurtosis analyses]. Colored overlays represent statistically significant differences for healthy control > advanced glaucoma (threshold-free cluster enhancement-corrected, p<0.05) in the OR of each diffusion parametric map. **(B)** ROI analyses pertinent to each map in Fig. 1A showed significant group differences (*: p < 0.05, **: p < 0.01) or significant linear trends (#: p < 0.05, ##: p < 0.01, ###: p < 0.001; n.s.: not significant) in the left or right OR between healthy control, early glaucoma, and advanced glaucoma subjects, consistent with the laterality and significance of the TBSS counterparts.

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Poster

P352. Biomarkers of Disease and Injury: Human Studies

Location: Virtual Only

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Program #/Poster #: P352.05

Topic: C.10. Brain Injury and Trauma

Support: P20GM103434 to the West Virginia IDeA Network for Biomedical Research Excellence
R24 MH106057-03
NSF IIP-19295

Title: Bedside PET imager: potential for research and point-of-care imaging

Authors: *J. BREFCZYNSKI-LEWIS[1], A. STOLIN[1], N. SIVA[1], C. GLOVER[1], S. RAJAGOPALAN[1], K. NOTT[1], S. CHANDI[1], T. WUEST[1], J. W. LEWIS[2], G. MORANO[1], S. MAJEWSKI[3];
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Abstract: Hospital and research PET are typically done at specialized facilities with a body-dedicated PET imager that require a supine position. Multiple head-dedicated PET brain imagers have been developed in recent years, but none have allowed patients or research subjects to remain in their own bed or chairs, or stand upright. We have developed a brain dedicated PET system that is self-contained with a rolling cart and arm that supports the imager in multiple positions. We tested the imager on both phantom (NEMA and Hoffman brain) and in human participants. Methods. 16 Hamamatsu TOF-capable modules were connected in a ring formation, with a diameter of 26.7cm, with the aim of accommodating most human heads, including the tubing for brain injury patients. Both NEMA NU-4 and Hoffman brain phantoms were tested using standard quality control testing parameters (Stolin et al. 2019). The imager was then placed in a custom constructed encasement that supported the imager at multiple head angles. The encasement was attached to an overhead height-adjustable arm that was connected to a cart that housed the computer with imaging processing unit. Four clinical PET-scan scheduled participants were seated comfortably, upright on a chair. They were given 1-2 millicuries of fluorodeoxyglucose and were then scanned by the Bedside PET scanner for 5 minutes of the first scan after receiving 10% of the injection. After uptake of the full clinical dose (90min) the patients receive another 5-minute scan. Results. NEMA NU-4 “rat” phantom tests revealed a spatial resolution of 2.3 mm at 5 mm from the center of the scanner, with a peak sensitivity of 1.28% and temporal TOF resolution of 462 ps. Image quality of the Hoffman brain revealed clear delineation of sulci and brain structures. For the patient imaging, brain images were collected, but image quality was affected by random/scatter from the whole body dose as well as

motion artifact. Conclusion and future directions. The bedside PET imaging system reveals that a workable imager can be made from commercially available PET modules. Image quality using phantoms was promising, but logistics to prevent head motion and to reduced body radiation interference with image quality. Head stabilizer options and lead shielding options will be explored. Bedside PET scanning would allow greater access of brain imaging to critical care patients who are at risk of stroke in supine position or due to transport to brain imaging facilities. It would also reduce contamination time needed for infectious patients and allow for smaller clinic imaging. As there is no need for specialized room shielding, research use in behavioral laboratories is facilitated.

Disclosures: J. Brefczynski-Lewis: None. **A. Stolin:** None. **N. Siva:** None. **C. Glover:** None. **S. Rajagopalan:** None. **K. Nott:** None. **S. Chandi:** None. **T. Wuest:** None. **J.W. Lewis:** None. **G. Morano:** None. **S. Majewski:** None.

Poster

P352. Biomarkers of Disease and Injury: Human Studies

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P352.06

Topic: C.10. Brain Injury and Trauma

Title: Electroencephalogram data-driven machine learning for traumatic brain injury and stroke classification

Authors: *M. CAIOLA[1], N. VIVALDI[2], M. YE[1];
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Abstract: Background: Traumatic brain injury (TBI) presents a significant challenge affecting an estimated 2.5 million people annually. Current clinical scores can classify TBI by severity but not with enough sensitivity to detect mild TBI or monitor progression. Therefore, ongoing efforts seek alternative clinical assessment tools for TBI. EEG has advantages of being non-invasive, easy-to-use, portable, and cost effective. However, when applied to TBI research, EEG yields mixed results in the literature with some studies showing significant differences in EEG-based power spectra data between mild TBI and normal groups, while others report no such distinction. Due to the inherent complexity of TBI, including the absence of consensus on biomarkers, underlying relationships between data, and patient-to-patient variability, big data analytics have the potential to make determinations about population characteristics that would otherwise be too difficult/impossible to manually identify. By comparing EEGs recorded between normal, TBI, and stroke patients, we can show unique and novel differences between the different disease brain states. Methods: We evaluated the performance of commonly used supervised machine learning algorithms in the classification of patients with TBI history (N=142) from those with stroke history (N=165) and normal EEG (N=105). Support vector machine (SVM) and K-nearest

neighbors (KNN) models were generated with a diverse feature set (selected using various feature section techniques) from Temple EEG Corpus for three-class classification of TBI, stroke and normal subjects. This was then followed by a deep-learning Convolutional Neural Net (CNN) approach using the same pool of populations but nearly 10 times the EEG samples. Results: Accuracies of 0.85 and 0.71 were reached in Cross Validation (CV) and Independent Validation (IV) respectively. Overall, linear discriminant analysis (LDA) feature selection and SVM models consistently performed well in both CV and IV. Compared to normal control, both TBI and stroke patients showed an overall reduction in coherence and relative power spectral density in delta frequency, and an increase in higher frequencies. But stroke patients showed a greater degree of change and had additional global decrease in theta power. Our study suggests that EEG data-driven machine learning can be a useful tool for TBI classification and can potentially provide specificity to separate different neurological conditions.

Disclosures: M. Caiola: None. **N. Vivaldi:** None. **M. Ye:** None.

Poster

P379. Pain Mechanisms: Nociceptors: Ion Channels II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P379.01

Topic: D.02. Somatosensation

Support: NIH Grant NINDS R01 NS076614
Weill Neurohub

Title: The role of epidermal cells in Drosophila melanogaster nociception

Authors: *S. S. MALI[1], J. YOSHINO[2], C. R. WILLIAMS[2], J. Z. PARRISH[2], D. M. BAUTISTA[1,2];
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Abstract: For an organism to survive, it needs to rapidly detect harmful stimuli in the environment and respond appropriately. Our studies, and others, have shown that epithelial cells, our first point of contact with the environment, work in concert with sensory neurons to mediate responses to noxious stimuli. We find that epidermal cells in Drosophila melanogaster larvae are physically and functionally coupled to somatosensory neurons, exhibit heterogeneity in their sensitivity to mechanical and thermal stimuli, and activation of epidermal cells modulates behavioral responses to noxious stimuli. Ultrastructural imaging studies show that epidermal cells in Drosophila larvae ensheath nociceptive class IV dendrite arborization (c4da) neurons, and that this anatomical coupling modulates nociception. Optogenetic stimulation of epidermal cells evokes calcium transients in somatosensory neurons and elicits a stereotyped nocifensive behavioral response. Using single cell calcium imaging and electrophysiology, we have found

that epidermal cells are intrinsically mechano- and thermosensitive. RNA-sequencing analysis demonstrates that Drosophila epidermal cells express a repertoire of sensory channels, and thus, we have set out to identify key signaling molecules for sensory transduction in epidermal cells. This work will further our understanding of a highly evolutionarily conserved sensory system and offer new cellular and molecular targets for the urgent unmet need of pain therapeutics.

Disclosures: S.S. Mali: None. **J. Yoshino:** None. **C.R. Williams:** None. **J.Z. Parrish:** None. **D.M. Bautista:** None.

Poster

P379. Pain Mechanisms: Nociceptors: Ion Channels II

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Program #/Poster #: P379.02

Topic: D.02. Somatosensation

Support: Funded by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM103436. (H.T.)

Title: The effect of TEA, 4-AP and in combination on primary sensory neurons in marine crustacean model

Authors: *H. TANNER[1], R. L. COOPER[2];
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Abstract: The channels for transmitting electrical activity along neurons is similar from squid to humans with sodium currents accounting for the upstroke of an action potential and potassium channels for the rapid and delayed ionic flux for the repolarization. Pharmacological agents tetraethylammonium chloride (TEA) and 4-aminopyridine (4-AP) block different subsets of voltage gated potassium (K+) channels. The chordotonal organs in crab limbs are a model of proprioceptive sensation and have rapidly- and slowly-adapting sensory neurons. Since 4-AP is used clinically for amyotrophic lateral sclerosis (ALS) treatments, a better understanding of its action on proprioceptive models can aid in understanding the potential effects for mammalian systems. To assess the action of these blockers on the function of proprioceptive sensory neurons, the neurons were evoked by movements associated with the joint while applying these compounds individually as well as in combination. Both 4-AP and TEA decreased activity individually, as well as when combined together. Full function was not returned in most preparations after the 4-AP and TEA were washed out both individually and in combined settings. Thus, the potassium channels are sensitive to both blockers in this marine crustacean model.

Disclosures: H. Tanner: None. **R.L. Cooper:** None.

Poster

P379. Pain Mechanisms: Nociceptors: Ion Channels II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P379.03

Topic: D.02. Somatosensation

Support: NIH R21MH122987
NIH R01GM140130
the Research Start-Up Funds from the School of Neuroscience at Virginia Tech

Title: Ionotropic Receptor-dependent cool cells control the transition of temperature preference in Drosophila larvae

Authors: J. TYRRELL, J. WILBOURNE, A. OMELCHENKO, J. YOON, *L. NI;
Virginia Tech., Blacksburg, VA

Abstract: Temperature sensation guides animals to avoid temperature extremes and to seek their optimal temperatures. The larval stage of Drosophila development has a dramatic effect on temperature preference. While early-stage Drosophila larvae pursue a warm temperature, late-stage larvae seek a significantly lower temperature. Previous studies suggest that this transition depends on multiple rhodopsins at the late larval stage. Here, we show that early-stage larvae, in which dorsal organ cool cells (DOCCs) are functionally blocked, exhibit similar cool preference to that of wild type late-stage larvae. The molecular thermoreceptors in DOCCs are formed by three members of the Ionotropic Receptor (IR) family, IR21a, IR93a, and IR25a. Early-stage larvae of each Ir mutant pursue a cool temperature, similar to that of wild type late-stage larvae. At the late larval stage, DOCCs express decreased IR proteins and exhibit reduced cool responses. Importantly, late-stage larvae that overexpress IR21a, IR93a, and IR25a in DOCCs exhibit similar warm preference to that of wild type early-stage larvae. These data suggest that IR21a, IR93a, and IR25a in DOCCs navigate early-stage larvae to avoid cool temperatures and the reduction of these IR proteins in DOCCs results in animals remaining in cool regions during the late larval stage. Together with previous studies, we conclude that multiple temperature-sensing systems are regulated for the transition of temperature preference in fruit fly larvae.

Disclosures: J. Tyrrell: None. **J. Wilbourne:** None. **A. Omelchenko:** None. **J. Yoon:** None. **L. Ni:** None.

Poster

P379. Pain Mechanisms: Nociceptors: Ion Channels II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P379.04

Topic: D.02. Somatosensation

Support: NSF Grant# 11520-02-412009377

Title: Long isoforms of the mechanoreceptor pezo-1 control feeding in C. elegans

Authors: *K. HUGHES[1], A. SHAH[1], X. BAI[2], J. ADAMS[1], R. BAUER[2], J. JACKSON[1], C. BAINBRIDGE[1], E. HARRIS[1], A. FICCA[1], P. FREEBAIRN[1], S. MOHAMMED[1], E. M. FERNANDEZ[3], M. BROCCO[3], W. STEIN[1], A. G. VIDAL-GADEA[1];
[1]Illinois State Univ., Normal, IL; [2]Natl. Inst. of Diabetes and Digestive and Kidney Diseases, Natl. Inst. of Hlth., Bethesda, MD; [3]Lab. de neurociencias, Inst. De Investigaciones Biotecnológicas Rodol, La Matanza, Argentina

Abstract: Two PIEZO mechanosensitive cation channels, PIEZO1 and PIEZO2, have been identified in mammals, where they are involved in numerous sensory processes. While structurally similar, PIEZO channels are expressed in distinct tissues and exhibit unique properties. How different PIEZOs transduce force, how their transduction mechanism varies, and how their unique properties match the functional needs of the distinct tissues where they are expressed remains unknown. The nematode Caenorhabditis elegans has a single PIEZO ortholog (pezo-1) predicted to have twelve isoforms. While all isoforms share many transmembrane domains, they differ in the number shared, particularly in those underlying the differences between PIEZO1 and PIEZO2 in mammals. Here we use translational and transcriptional reporters to show that long pezo-1 isoforms are selectively expressed in mesodermally derived tissues (such as muscle and glands). We show that pharyngeal muscles, glands, and valve all rely on long pezo-1 isoforms to respond appropriately to the presence of food. We found that gland cell activation is modulated by food presence and density. However, RNAi against long isoforms of pezo-1 resulted in gland cells with reduced activity that did not significantly respond to food. The number of pezo-1 isoforms in C. elegans, their differential pattern of expression, and their role in experimentally tractable processes make this an attractive system to investigate the molecular basis for functional differences between members of the PIEZO family of mechanoreceptors.

Disclosures: K. Hughes: A. Employment/Salary (full or part-time):; Illinois State University. **A. Shah:** None. **X. Bai:** A. Employment/Salary (full or part-time):; National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health. **J. Adams:** A. Employment/Salary (full or part-time):; Illinois State University. **R. Bauer:** A. Employment/Salary (full or part-time):; Northwestern University. **J. Jackson:** None. **C. Bainbridge:** A. Employment/Salary (full or part-time):; University of Rochester. **E. Harris:** None. **A. Ficca:** None. **P. Freebairn:** None. **S. Mohammed:** None. **E.M. Fernandez:** None. **M. Brocco:** None. **W. Stein:** A. Employment/Salary (full or part-time):; Illinois State University.

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Poster

P379. Pain Mechanisms: Nociceptors: Ion Channels II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P379.05

Topic: D.02. Somatosensation

Support: NIH R01GM140130
NIH R21MH122987
Research Start-Up Funds from the School of Neuroscience at Virginia Tech

Title: Ionotropic Receptor-dependent dorsal organ warm cells mediate warm sensing in Drosophila larvae

Authors: *A. OMELCHENKO, J. TYRRELL, L. NI;
Sch. of Neurosci., Virginia Tech., Blacksburg, VA

Abstract: Temperature sensation is critical for animals to avoid thermal extremes, seek optimal temperatures, and regulate body temperature. It is particularly important for small animals, such as fruit flies, which rely on the ambient temperatures for homeostasis. Though dorsal organ cool cells (DOCCs) and their function in cool sensing have been extensively studied in fly larvae, the cellular and molecular aspects of warm sensing are largely unknown. Here, we identified two warm-activated neurons in the vicinity of DOCCs, named dorsal organ warm cells (DOWCs), that are critical for choosing optimal temperatures within a specific temperature range. Three members of the Ionotropic Receptor (IR) family, IR25a, IR93a, and IR92a, in DOWCs are required for the warm responsiveness of DOWCs and the thermotactic behavior. This study identified a new set of neurons that depend on IRs to respond to warm stimuli and control larval warm sensing in innocuous temperatures. Interestingly, the previously identified ammonia receptor IR92a is a component of the warm receptor, suggesting that a tuning IR can pair with different sets of IR coreceptors for distinctive functions.

Disclosures: A. Omelchenko: None. **J. Tyrrell:** None. **L. Ni:** None.

Poster

P379. Pain Mechanisms: Nociceptors: Ion Channels II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P379.06

Topic: D.02. Somatosensation

Support: NIH Grant R01 NS115209-01

Title: Transient and steady-state spiking response of the Drosophila noxious cold-sensing neurons

Authors: *A. SAKURAI, N. MAKSYMCHUK, D. N. COX, G. S. CYMBALYUK;
Neurosci. Inst., Georgia State Univ., Atlanta, GA

Abstract: Temperature is one of the most critical environmental factors that affect the life of insects. The ability to sense temperature and take appropriate action is crucial to their survival. This study investigated the mechanisms of cold temperature coding by the *Drosophila* larva Class III (CIII) neurons by applying electrophysiological and computational neuroscience methods.

Electrophysiological recordings revealed that, to a fast temperature drop, most CIII neurons responded by a peak of spiking rate followed by spike frequency adaptation at the steady cold temperature interval of stimulation. In contrast, most CIII neurons responded with a gradual increase of spiking rate to a slow temperature decrease. On average, the steady-state spiking rate increased at lower temperatures. Fitting the spiking rate at different temperatures with the Boltzmann's function revealed high variability in temperature of half-maximal activation. Cell-to-cell variation was also seen in the pattern of spiking activity: all CIII neurons showed tonic spiking activity during the cold stimulation, while about 50% of them, depending on the stimulus intensity, exhibited bursting activity. Bursting activity more often appeared within 20 seconds after the onset of the fast cold stimulus, whereas at slow temperature decrease, bursting occurs at lower rates and broadly distributes across the whole temperature range. There was a significant positive correlation between the burst frequency and the rate of change in temperature.

Based on transcriptomic data from *Drosophila* CIII neurons, we developed a computational model implementing basic TRP current kinetics with temperature-dependent activation and Ca^{2+}-dependent inactivation. The model suggested that the kinetics of a TRP current may contribute to mechanisms underlying the phasic-tonic responses of CIII neurons and coding the rate of temperature change and cold temperature value. The model also exhibited both cold-evoked activity regimes, tonic spiking, and bursting. These regimes were determined by the kinetics of TRP current and parameters of pattern-generating subsystems of the model, including Ca^{2+} activated K^{+} currents and voltage-gated Ca^{2+} channels.

Together the results indicate that the steady temperature value was encoded by the tonic spiking activity, whereas accumulation of bursting activity at the time of a rapid temperature drop suggests that CIII neurons may encode the rate of temperature decrease by bursting activity.

Disclosures: A. Sakurai: None. **N. Maksymchuk:** None. **D.N. Cox:** None. **G.S. Cymbalyuk:** None.

Poster

P379. Pain Mechanisms: Nociceptors: Ion Channels II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P379.07

Topic: D.02. Somatosensation

Support: NSERC
CIHR
University of Saskatchewan, College of Medicine

Title: Role of Cystic Fibrosis Transmembrane conductance Regulator in chloride homeostasis in swine sensory neurons

Authors: N. HENAO-ROMERO, J. P. IANOWSKI, ***V. A. CAMPANUCCI**;
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Abstract: Cystic Fibrosis (CF) is a common fatal disorder associated with mutations of recessive inheritance in the gene that encodes for the cystic fibrosis transmembrane regulator (CFTR) protein. Although traditionally considered a deadly pediatric disease due to respiratory failure and gastrointestinal (GI) complications, current advances in treatment and management have increased the average life expectancy of CF patients in Canada to 51 years of age. Therefore, now adult CF patients are exposed to previously unknown chronic complications of the CF pathology, such a peripheral neuropathy. In addition, considerable evidence shows that CFTR is not only expressed in epithelial cells as initially thought, but it is also widely expressed in the nervous system. However, the role of CFTR in neurons and its potential contribution to the CF pathology remains to be explored. In the current study, we investigated the cellular effects of lack of CFTR function in dorsal root ganglia (DRG) neurons. We quantified chloride levels in DRG primary cultures from wild-type (WT) and CFTR -/- swine using a chloride sensitive dye (i.e., MQAE) and perforated patch-clamp electrophysiology. In addition, we used the CFTR inhibitor 172 (CFTR (inh)-172) and current-clamp electrophysiology to investigate the effect of pharmacological inhibition of CFTR, versus the lack of its expression, in DRG neuron function. Our findings indicate that DRG neurons from CFTR -/- swine had a lower intracellular chloride concentration than WT neurons. We also observed that pharmacological inhibition of CFTR for 48 hr had no effect on DRG neuronal excitability or on the GABA-mediated modulation of action potential firing in DRG neurons. Taken together, our data suggest that CFTR contributes to chloride homeostasis in DRG neurons, possibly acting in combination with the Na-K-Cl co-transporter (NKCC1), which drives chloride influx. Thus, our data show for the first time that lack of CFTR function may alter DRG neuron function and contribute to the development of peripheral neuropathy in CF patients.

Disclosures: N. Henao-Romero: None. **J.P. Ianowski:** None. **V.A. Campanucci:** None.

Poster

P412. Barrel Cortex: Cells, Function, Circuitry

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P412.01

Topic: D.04. Somatosensation – Touch

Support: NIH Grant NS116604
NIH T32 Training Grant GM132494
Grant P20GM103434
Grant U54GM104942

Title: Identifying somatostatin interneuron subsets by combining intersectional genetics, retrograde labeling, and immunohistochemistry

Authors: *R. HOSTETLER, H. HU, A. AGMON;
Dept of Neurosci., West Virginia Univ., Morgantown, WV

Abstract: Somatostatin (SOM) inhibitory interneurons are the second largest inhibitory subtype in the neocortex, but their considerable diversity has hampered attempts to understand their role in cortical circuits underlying sensory, motor and cognitive functions. Subsets of SOM cells differ in their layer-specific axonal projections, marker protein expression, and electrophysiological properties, but most previous studies used transgenic approaches which target all SOM cells indiscriminately. There is therefore an acute need for well-validated tools to identify and target distinct subsets of SOM cells. To address this need we used intersectional genetics, and validated this approach with retrograde labeling from layer 1 (L1), immunohistochemical staining in fixed tissue, and ex-vivo electrophysiological recordings (see companion poster).We crossed the SOM-Flp mouse line with lines expressing Cre recombinase driven by SOM subset-specific promoters: nicotinic receptor alpha 2 subunit (Chrna2)-Cre, calretinin (CR)-Cre and prodynorphin (pDyn)-Cre, and with a dual reporter (RC::FLTG) which expresses GFP in Cre+/Flp+ cells and tdTomato in Cre-/Flp+ cells. L1-projecting cells were retrogradely labeled by placing a Fast Blue (FB) dyed filter paper on the pial surface of the somatosensory cortex followed by 24 hour survival. Fixed brain sections were then stained with antibodies to determine expression of the marker proteins CR, calbindin (CB) and neuropeptide Y (NPY). GFP and tdTomato-expressing cells within the FB-labeled region were counted by layer and scored as FB+ or FB- and as antibody+ or antibody-. Male and female mice (age 6-24 weeks) were used, N=3 per genotype.The majority of SOM cells in L2/3 and L5 were FB+ (i.e. L1-targeting), while the majority in layers L4 and L6 were FB-. The vast majority of Chrna2-Cre cells were in L5 and most were FB+, CB+, CR- and NPY-. The majority of CR-Cre cells were in L2/3 and L5, were FB+, and were CR+, CB+ and NPY+. The majority of pDyn-Cre cells were in L4 and L5 and were FB- in L4, but ~50% were FB+ in L5. Most FB- pDyn-Cre cells were CB-,

NPY-, and CR-, while most FB+ pDyn-Cre cells were CB+, and some were CR+ and NPY+. We conclude that CR-Cre and Chrna2-Cre SOM cells represent two distinct, non overlapping L1-projecting SOM subsets in L5. PDyn-Cre SOM cells contain at least two distinct, non overlapping groups, cells that are L1-projecting, CB+ and occasionally CR+, and cells that are non L1-projecting and are CB- and CR-. The former group likely includes the CR-Cre subset, and the latter most likely corresponds to the previously described X94 subset.

Disclosures: R. Hostetler: None. **H. Hu:** None. **A. Agmon:** None.

Poster

P412. Barrel Cortex: Cells, Function, Circuitry

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P412.02

Topic: D.04. Somatosensation – Touch

Support: NIH Grant NS116604

Title: Electrophysiological characterization of three non-overlapping layer 5 SOM interneuron subtypes

Authors: H. HU, R. HOSTETLER, ***A. AGMON**;
Neurosci., West Virginia Univ., Morgantown, WV

Abstract: Somatostatin-containing (SOM) neurons are the second largest group of inhibitory neocortical interneurons; however efforts to decipher their roles in cortical circuits underlying sensory, motor and cognitive functions have been hampered by their considerable diversity. SOM interneurons vary in their layer-specific axonal projections, electrophysiological fingerprints, and marker protein expression (e.g. cell-surface receptors and neuropeptides). Especially diverse is the SOM population in layer 5, which consists of both layer 1-targeting and layer 4-targeting SOM cells. Previous studies from our lab and others have examined SOM interneuron diversity using transgenic mouse lines with subset-specific expression of GFP, and more recently by intersectional genetics; however most studies to-date have used mouse Cre lines which target all SOM interneurons indiscriminately, leading to confusing and at times contradictory results. Thus, well-validated genetic tools to identify and target the major SOM interneuron subsets in-vivo and ex-vivo are acutely needed. Here we used triple-transgenic mouse lines to target 3 major non-overlapping layer 5 SOM subsets for electrophysiological recordings, and show that the three subsets can be reliably identified by their electrophysiological parameters and firing patterns. We crossed SOM-Flp mice with mice expressing Cre driven by promoters for the marker proteins calretinin (CR), Chrna2 and prodynorphin (pDyn). Further crossing with the RC::FLTG reporter resulted in GFP in Cre-expressing SOM cells and tdTomato in all other SOM cells. For validation, we also crossed

Chrna2-Cre and pDyn-Cre mice with X94 and X98 mice in which GFP is expressed, respectively, in subsets of layer 4-targeting and layer 1-targeting SOM cells. We then conducted single and dual ex-vivo whole-cell recordings from GFP and/or td-Tomato-expressing neurons, in 1-3 months old male and female mice. We found that Chrna2-Cre, CR-Cre and X94 SOM cells in layer 5 have largely non-overlapping and highly distinct electrophysiological fingerprints which should allow their reliable identification even without fluorescent protein expression. These subsets are also distinct in their axonal projection targets and marker protein expression, as shown in the companion poster. PDyn-Cre cells are a superset which contains the X94 subset. Beyond mere subtype characterization, this intersectional approach can be used to express inhibitory and excitatory opsins or calcium and voltage reporters in distinct subsets of SOM interneurons, enabling investigations of their roles in sensory perception, behavior and learning.

Disclosures: H. Hu: None. **R. Hostetler:** None. **A. Agmon:** None.

Poster

P412. Barrel Cortex: Cells, Function, Circuitry

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P412.03

Topic: D.04. Somatosensation – Touch

Support: NIH 1 F32 NS114327
NIH 1 R37 NS092367

Title: Sensory and action-related responses of VIP interneurons in mouse whisker S1 during goal-directed behavior

Authors: *D. L. RAMAMURTHY, A. CHEN, P. C. HUANG, P. BHARGHAVAN, G. KRISHNA, K. CASALE, D. E. FELDMAN;
Helen Wills Neurosci. Inst. and MCB Dept., UC Berkeley, Berkeley, CA

Abstract: Vasoactive intestinal peptide-expressing (VIP) interneurons have emerged as an important GABAergic cell type for regulating cortical activity via disinhibition of pyramidal (PYR) cells. In primary somatosensory cortex (S1), VIP neurons respond to motor and neuromodulatory signals during active whisking and locomotion to promote PYR cell firing, but the function of VIP cells in sensory processing of whisker tactile stimuli is not well understood. VIP neurons respond to passive whisker deflections, but their stimulus selectivity and activation by other behaviorally relevant signals like reinforcement is unknown. Therefore, we developed a Go/NoGo whisker detection task with a post-stimulus delay period that separates sensory-driven activity from action- or reward-related neural signals during goal-directed behavior. Vip-cre; Ai162 (TIGRE2.0-GCaMP6s) mice were trained to lick to deflection of a randomly chosen whisker (Go trials) to receive a reward, but not lick on trials in which no whisker was deflected

(NoGo trials, which were neither rewarded nor punished). We used 2-photon calcium imaging to measure activity of L2/3 VIP neurons to goal-relevant whisker stimuli, licks, and rewards in behaving mice. ~70% of imaged VIP cells showed significant whisker responses. VIP cells exhibited whisker receptive fields. ~65% of whisker-responsive cells had a single best whisker (BW) that evoked a significantly stronger response than the next-best whisker (which evoked ~60% of BW response). Whisker tuning of VIP cells was slightly broader than for PYR cells. VIP cells showed an intermixed tuning organization in which only ~40% of VIP cells in a given anatomical whisker column were tuned for the columnar whisker. This is similar to the salt-and-pepper organization observed for PYR cells. In addition, most VIP cells (~60%) showed strong lick-evoked activity, which was on average ~40% of the magnitude of the best whisker-evoked response). On average, reward delivery on hit trials did not elevate VIP cell activity over that seen on false alarm trials, or during spontaneous licking. Thus, VIP interneurons in S1 represent both sensory and motor (lick) events during goal-directed behavior. Our results suggest that VIP interneurons contribute to stimulus feature selectivity in S1, in addition to their known role in mediating motor influences.

Disclosures: D.L. Ramamurthy: None. **A. Chen:** None. **P.C. Huang:** None. **P. Bharghavan:** None. **G. Krishna:** None. **K. Casale:** None. **D.E. Feldman:** None.

Poster

P412. Barrel Cortex: Cells, Function, Circuitry

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P412.04

Topic: D.04. Somatosensation – Touch

Support: AFOSR GRANT13022629

Title: Layer-specific regulation of SST inhibition in mouse barrel cortex during whisker-dependent sensory learning

Authors: *E. PARK, D. A. KULJIS, A. L. BARTH;
Biol. Sci. Dept., Carnegie Mellon Univ., PITTSBURGH, PA

Abstract: Somatostatin (SST)-expressing GABAergic neurons comprise a major class of inhibitory interneurons in the cerebral cortex. Previous studies have suggested that inhibition from SST neurons may be regulated during sensory experiences and learning. However, whether changes in SST neurons are state-dependent or manifested as changes in synaptic weights, as well as the cellular target of these changes, remain unclear. Here we investigated whether SST output onto pyramidal (PYR) neurons in the primary somatosensory cortex was altered during sensory association training, using analysis of SST-IPSCs in acute brain slices from trained animals. Because downregulation of SST interneurons is hypothesized to be critical for gating

synaptic plasticity, we hypothesize that reduction in SST-neuronal output might facilitate the potentiation of excitatory synapses that has been detected in previous studies. We found that SST-neuronal output is reduced on layer 2/3 PYR neurons but not on layer 5 PYR neurons after sensory association training. Furthermore, the reduction of SST input was not apparent when the water reward was decoupled from the whisker stimulation. Our results suggest that the depression of SST output onto PYR neurons is a layer-specific modification selectively engaged during reinforcement learning but not sensory exposure.

Disclosures: E. Park: None. **D.A. Kuljis:** None. **A.L. Barth:** None.

Poster

P412. Barrel Cortex: Cells, Function, Circuitry

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P412.05

Topic: D.04. Somatosensation – Touch

Support: NIH/NNDS R01 Grant NS107599
Whitehall Foundation Grant 2017-05-71

Title: Cortical Feedback Activity Under Anesthesia is Pathway Dependent

Authors: *K. ARULJOTHI[1], A. LAM[2], E. ZAGHA[1];
[1]Dept. of Psychology, [2]Biomed. Sci., Univ. of California Riverside, Riverside, CA

Abstract: 'Top-down processing' refers to the use of prior knowledge and internal context to bias sensation, perception, and action. Studies of cortical anatomy and physiology support that top-down processing is mediated, in part, by cortical feedback pathways, which connect higher order to lower order cortical regions. Given the importance of top-down processing during wakefulness, a long standing assumption with some experimental evidence is that cortical feedback is not active under anesthesia. In this study, we measure the spontaneous activity of two cortical feedback pathways during slow wave anesthesia. We demonstrate that under anesthesia the retrosplenial cortex to primary visual cortex feedback pathway is not active, yet the whisker motor cortex to whisker primary somatosensory cortex feedback pathway is active. These findings suggest that the functions of cortical feedback are not limited to wakefulness. Ongoing studies are attempting to determine the mechanisms underlying these activity differences.

Disclosures: K. Aruljothi: None. **A. Lam:** None. **E. Zagha:** None.

Poster

P412. Barrel Cortex: Cells, Function, Circuitry

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P412.06

Topic: D.04. Somatosensation – Touch

Support: K99-NS096108
R00-NS096108
R00-NS096108-S1

Title: Short-term dynamics of long-range corticocortical synapses revealed by selective optical stimulation

Authors: *L. E. MARTINETTI[1], K. E. BONEKAMP[2], D. M. AUTIO[2], S. R. CRANDALL[2]; [1]Dept. of Physiol., Michigan State Univ. Neurosci. Program, Lansing, MI; [2]Dept. of Physiol., Michigan State Univ., East Lansing, MI

Abstract: Synapses are continually regulated by their own activity. In the neocortex, direct interactions between cortical areas play a central role in cognitive function, but the dynamic regulation of these long-range corticocortical synapses by activity and their impact on a postsynaptic target neuron is unclear. Here, we use an optogenetic strategy to study the connections between mouse somatosensory and motor cortex. We found that short-term synaptic facilitation was strong in both corticocortical synapses, resulting in far more sustained responses than local intra-cortical and thalamocortical connections. This facilitation was dependent on the presynaptic calcium sensor synaptotagmin-7 and altered by several optogenetic approaches. Recordings revealed that during repetitive activation, the short-term dynamics of corticocortical synapses enhanced the excitability of layer 2/3 pyramidal neurons, increasing the probability of spiking with activity. Furthermore, the properties of the connections linking primary with secondary somatosensory cortex resemble those between somatosensory-motor areas. These results reveal a synaptic mechanism by which corticocortical projections may mediate specific changes in cellular excitability over relatively extended periods.

Disclosures: L.E. Martinetti: None. **K.E. Bonekamp:** None. **D.M. Autio:** None. **S.R. Crandall:** None.

Poster

P412. Barrel Cortex: Cells, Function, Circuitry

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P412.07

Topic: D.04. Somatosensation – Touch

Support: NIH R37 NS092367

Title: Structured dynamics of the whisker map in layer 2/3 of somatosensory cortex in awake, behaving mice

Authors: *H. WANG, D. E. FELDMAN;
Helen Wills Neurosci. Inst., Univ. of California, Berkeley, Berkeley, CA

Abstract: In sensory cortex, single neurons exhibit spontaneous tuning instability that appears locally random yet remains bounded within mean sensory map topography. How tuning instability is organized within maps is unknown. We used 2-photon Ca^{2+} imaging to characterize spontaneous tuning dynamics in L2/3 pyramidal (PYR) cells in whisker somatosensory cortex (S1) of mice performing a whisker-attentive behavioral task. Neurons were sharply tuned for one or a few whiskers, and cells tuned to different whiskers were intermixed in a salt-and-pepper fashion. In each column, ~50% of cells were tuned for the columnar whisker (CW) and 50% for nearby non-columnar (non-CW) whiskers. To assess tuning stability, we imaged longitudinally in Drd3-Cre/TIGRE2.0-GCaMP6s mice, which stably express Ca^{2+} sensor in L2/3 PYR cells, over 4 sessions 4-7 days apart (total of 2-3 weeks of imaging). Whisker tuning was highly dynamic, with 29%, 36%, and 43% of neurons significantly changing whisker tuning over 1, 2, and 3-session intervals. In addition, many neurons lost and gained sensory responsiveness across days. Tuning instability was structured within the map: CW-tuned neurons exhibited relatively stable tuning, while 48% of non-CW tuned neurons changed whisker tuning over 3 sessions. The most sharply tuned neurons also tended to maintain constant tuning. Tuning dynamics persisted when whiskers were chronically paralyzed with Botox, suggesting that active whisker experience does not drive tuning dynamics. Thus, the whisker map in L2/3 combines a stable columnar core and a highly dynamic, non-columnar surround. This organization may explain how S1 cortex balances flexibility and stability of neural codes and selects optimal representations to perceive the complex environment.

Disclosures: H. Wang: None. **D.E. Feldman:** None.

Poster

P429. Taste III

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P429.01

Topic: D.05. Olfaction and Taste

Support: RO1 DC06666
M.R. Bauer Fellowship

Title: Bitter Taste Avoidance is Dependent on Olfactory Cues

Authors: *S. SCOTT, B. T. STONE, J.-Y. LIN, D. B. KATZ;
Brandeis Univ., Waltham, MA

Abstract: Consuming beneficial substances while rejecting unsafe ones is an evolutionarily-critical task. Both chemosensory systems, olfaction and gustation, work in a highly-integrated manor to guide this behavior. In the lab, these sensory systems are often disambiguated with specific experimental assays, such as the Brief Access Task (BAT), which quantifies a taste's hedonic value while controlling for other sensory variables like smell. It records licking behavior in response to blind, randomized presentations of nonvolatile and purportedly-odorless tastant solutions, which should prevent the animal from identifying the presented solutions prior to tasting. However, previous results found that rats avoid quinine (QHCl) at much higher rates than other presented tastants ($F(4, 79) = 53.9$, $p<.05$), suggesting they may identify tastants prior to licking. We hypothesize that rats either employ a chemosensory tag to mark lick spouts as they drink, or they are perceiving the olfactory characteristics of our nonvolatile and supposedly odorless tastants. To investigate, we randomly alternated delivery of vials of sucrose and QHCl differing number of times per vial, with and without a fan blowing. If avoidance behavior is cued by a pheromonal marker, avoidance would increase over the duration of a testing session as the tag was built up whereas if rats can simply smell the odorless tastants, avoidance should be reduced in fan-on trials. Our task-manipulation experiment demonstrates that avoidance does not scale with the number of times a vial has previously been presented during a testing session ($t(6) = 1.12$, $p>.05$). We show a significant difference in avoidance with and without a fan present ($F(1, 3) = 4.6$, $p < 0.05$)—indicating that QHCl avoidance behavior is likely due to rats perceiving olfactory characteristics of the QHCl solution. In conclusion, we demonstrate that male Long Evans rats can perceive olfactory elements of the commonly-used quinine-hydrochloride tastant solution and use those elements to guide decisions to avoid sampling behavior in a Brief Access Task.

Disclosures: S. Scott: None. **B.T. Stone:** None. **J. Lin:** None. **D.B. Katz:** None.

Poster

P429. Taste III

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P429.02

Topic: D.05. Olfaction and Taste

Support: NIH Grant 1 F31 NS108506-01

Title: Strong Aversive Conditioning Triggers a Long-Lasting Generalized Aversion

Authors: *R. RAMOS[1], C.-H. WU[2], G. TURRIGIANO[3];
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Abstract: All learning is a balance between the specific details of a memory and what can be generalized in future scenarios. While some degree of generalization can endow an animal with cognitive flexibility, persistent over-generalization is associated with pathological conditions, such as post-traumatic stress disorder. Currently, what the different forms of synaptic plasticity are that work to shape this balance and how they do so remains incompletely understood. Our previous experiments demonstrated that following Conditioned Taste Aversion (CTA) learning, a robust form of associative learning dependent on Hebbian mechanisms, animals formed a generalized aversion that, over the course of many hours, became specific to the conditioned tastant through synaptic downscaling of excitatory synapses onto the conditioning-active ensemble. Here, we characterize a different behavioral phenotype: a long-duration generalized aversion persisting at least two-weeks post-conditioning. Following just a single strong CTA conditioning event (0.30M LiCl), animals form a generalized aversion that can be measured at 3, 7, 10- and 14-days post-conditioning. Repeated exposure to the generalized tastant results in extinction of the generalized aversion. Interestingly, this generalized aversion and its attenuation do not impact the acquisition and maintenance of a specific taste aversion to the conditioned tastant, suggesting distinct mechanisms or sites of plasticity. We hypothesize that this persistent generalized state arises from experience-dependent gating of homeostatic plasticity. Currently, electrophysiological experiments are underway to record from the conditioning-active neural ensemble in the gustatory cortex of animals during the formation and reversal of the persistent long-lasting generalized aversion.

Disclosures: R. Ramos: None. **C. Wu:** None. **G. Turrigiano:** None.

Poster

P429. Taste III

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P429.03

Topic: D.05. Olfaction and Taste

Support: NIDCD Funding DC006666
NIDCD Funding DC016706

Title: Assessment of critical taste response to monosodium glutamate (umami)

Authors: *L. LIN[1], J.-Y. LIN[2], D. B. KATZ[3];
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Abstract: Monosodium glutamate (MSG) is a naturally occurring amino acid. While often used to enhance food's flavor, the subjective taste experience of MSG has left the taste field wondering whether it should be considered as one of the core tastes or merely as a mixture (of sweet and salt). While past research has touched on this subject, until recently this question was out of reach. As shown in the research from our lab (and others), taste identity information is reflected/coded in the neural activity of the gustatory cortex (GC; the cortical region of the central gustatory pathway). To gain a better understanding of MSG processing, we therefore looked at the evoked GC identity response to MSG and compare it to other basic tastes or taste mixtures. In this study we used female Long-Evans rats unilaterally implanted with a multi-channel electrode to GC. In each 60-min testing session, the water-deprived rats actively sampled (i.e., lick) different tastes while GC neuronal activity was recorded. Using a taste battery of MSG (0.1M), sucrose (0.1M), NaCl (0.1M) and a mixture of sucrose and NaCl, we assessed the similarity of the MSG neural response to sweet and salty tastes. Our results show that, in single unit analysis, we found that neurons that responded to MSG also responded to sucrose or/and NaCl. In the populational analysis involving a Receiver Operating Characteristic (ROC) with the mean firing rate from each GC neuron as the dimensions, we found that the evoked GC ensemble activity of MSG is more similar to NaCl and mixtures than that of sucrose. This pattern of results suggests that in GC, the evoked response of MSG resembles salt-like stimuli. However, this does not exclude "a sucrose component" in MSG. Future research will seek to investigate whether the removal of the salt-component of MSG (by amiloride, a sodium blocker) will shift the GC response toward sucrose-like activity so that we can gain insight into how the interaction between salt and sweet elements modulates cortical MSG processing.
Funding: DC006666 and DC016706 from the National Institute of Deafness and Other Communication Disorders

Disclosures: L. Lin: None. **J. Lin:** None. **D.B. Katz:** None.

Poster

P429. Taste III

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Program #/Poster #: P429.04

Topic: D.05. Olfaction and Taste

Support: NIH Grant R21DC015759

Title: Regulation of licking responses by amygdala somatostatin expressing neurons that project to the nucleus of solitary tract

Authors: *J. J. BARTONJO[1], S. ST. JOHN[2], R. F. LUNDY[1];
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Abstract: Neural processing in the nucleus of the solitary tract (NST) is critical for concentration-dependent intake of normally preferred and avoided taste stimuli (e.g., hedonic responding); and is influenced by descending input from numerous forebrain regions. In one region, the central nucleus of the amygdala (CeA), a subpopulation of neurons that project to the NST expresses the neuropeptide somatostatin (Sst). The present study investigated whether this CeA/Sst-to-NST pathway contributes to concentration-dependent intake of sucrose and quinine hydrochloride (QHCl) solutions. We used virus-based optogenetic tools and laser light illumination to manipulate the activity of CeA/Sst neurons that project to the NST. During brief-access lick trials (5s) and light-induced inhibition of CeA/Sst-to-NST neurons, mice licked significantly more to our three highest concentrations of QHCl compared to control mice, while sucrose intake was unaffected. Interestingly, light-induced activation of this descending pathway did not influence licking of either sucrose or QHCl. These findings suggest that the CeA/Sst-to-NST pathway must be active for normal affective responding to an exemplary aversive taste stimulus.

Disclosures: J.J. Bartonjo: None. **S. St. John:** None. **R.F. Lundy:** None.

Poster

P429. Taste III

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Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P429.05

Topic: D.05. Olfaction and Taste

Support: NIH F31 DC019518

Title: Extrasynaptic $GABA_A$ receptors in the gustatory cortex and taste-dependent impulsive behavior

Authors: *P. YEVOO, A. FONTANINI, A. MAFFEI;
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Abstract: Impulsivity is a preexisting trait in most people. It can be advantageous; however, when excessive, it becomes maladaptive. Heightened impulsivity is a predictor and/or consequence of many neuropsychiatric conditions, including eating disorders. Binge eating is a dysfunctional pattern of feeding behavior that consists of consuming palatable foods rich in sweets and fats. It is the most prevalent disorder in the United States and a major driving cause of severe obesity - a condition that affects roughly 40% of adults and 18% of children and adolescents.
Studies show that the insular cortex's (IC) circuitry may be altered in binge eating disorders. The IC has been associated with impulsive actions and classified as a neurobiological gate to develop impulsivity-related disorders. The anterior IC – also known as the gustatory cortex (GC) – is also

involved in the processing of taste, the expectation of reward, and decision-making regarding food consumption. Here, we test the hypothesis that altered levels of tonic GABAergic inhibition in GC play a central role in mediating taste-dependent impulsive choices. Using the neurosteroid allopregnanolone, we investigate the contribution of tonic inhibition to GC circuit excitability and impulsive behavior (IB).
Neurosteroid-sensitive extrasynaptic $GABA_AR$s contain the δ subunit. To determine their expression in GC, we examined the patterns of expression of δ-$GABA_AR$s using immunohistochemistry and showed that these receptors are localized in GC. To examine the receptor's functionality, we utilized patch-clamp recordings from neurons in acute slices to test the magnitude of tonic inhibitory currents and found an increase in tonic currents following neurosteroid application. We then assessed the effect of local neurosteroid infusion on IB. We used the Go/No-Go task to measure impulsivity for food by evaluating the ability to inhibit licking to sucrose during the No-Go cue and Brief Access Test to examine changes in taste responsiveness. Our data show that infusions of neurosteroids modulate licking behavior and may alter taste sensitivity, supporting the hypothesis that GC plays a central role in controlling ingestive behaviors and that tonic inhibition is a likely mechanism for the control of licking to predictive cues.
This work provides novel insights into differences in neural network physiological properties underlying taste-dependent IB. A more precise understanding of the mechanisms underlying deficits of inhibitory control will provide novel biomarkers for the diagnosis, treatment, and prevention of impulsivity-related neuropsychiatric disorders improving the lives of patients and their families.

Disclosures: P. Yevoo: None. **A. Fontanini:** None. **A. Maffei:** None.

Poster

P429. Taste III

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Program #/Poster #: P429.06

Topic: D.05. Olfaction and Taste

Support: F32-DC018485
R01-DC015234

Title: A critical period for the development and expression of taste preference

Authors: *H. C. SCHIFF[1], J. F. KOGAN[1], M. ISAAC[1], A. FONTANINI[2], A. MAFFEI[3]; [1]Stony Brook University, Neurobio. & Behavior, Stony Brook, NY; [2]Dept. of Neurobio. & Behavior, SUNY Stony Brook, Stony Brook, NY; [3]Neurobio. and Behavior, SUNY-Stony Brook, Stony Brook, NY

Abstract: Taste preferences are critical for survival as they direct consumption towards the most nourishing food and away from dangerous substances. As animals transition from relying on their mother's milk to foraging for food, they experience an abundance of new tastes and acquire relevant information associated with consuming them. Although evidence from humans suggests that these early life experiences shape taste preferences, studies have not examined whether cortical regions processing gustatory information show plasticity in critical periods or remain plastic throughout life. Here we report, in mice, that taste experience at the time of weaning, but not in adulthood, affects preference for sweet tastes later in life. Using a brief access test, we determined that sucrose preference is enhanced following exposure to a variety of tastes at weaning compared to naïve mice exposed only to water and chow. The same exposure at 8 weeks of age did not affect sucrose preference. The change in sucrose preference did not depend on familiarity with sucrose. Within the gustatory cortex (GC), early exposure to tastants accelerated the association of parvalbumin (PV^+) neurons with perineuronal nets (PNNs) and increased the frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) onto excitatory neurons. Degrading PNNs with intra-GC infusions of chondroitinase ABC restored sensitivity to taste exposure in adults. Calcium imaging of GC PV^+ neurons is currently underway to examine their response to sucrose following exposure. These results point to the presence of a critical period when experience during the postnatal period induces long-term changes in taste preference and a central role for PV^+ neurons and their association with PNNs in the experience-dependent modulation of taste preference.

Disclosures: H.C. Schiff: None. **J.F. Kogan:** None. **M. Isaac:** None. **A. Fontanini:** None. **A. Maffei:** None.

Poster

P429. Taste III

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Program #/Poster #: P429.07

Topic: D.05. Olfaction and Taste

Support: DC006666
DC007703

Title: The contribution of the lateral hypothalamus to cortical palatability coding and behavior

Authors: *K. MAIGLER[1], D. B. KATZ[2];
[2]Dept Psychol, [1]Brandeis Univ., Waltham, MA

Abstract: Both the lateral hypothalamus (LH) and primary taste cortex (GC) are emphatically involved in the modulation of feeding behavior, but there is currently no evidence for how they interact with each other. GC ensembles enter a state that reflects the hedonic value of the taste

(palatability) as part of the taste-evoked response; this transition both predicts and drives consumption behaviors. LH also encodes palatability as part of the taste-evoked response with dynamics that dovetail nicely with those observed in GC. Both experimental and theoretical work on metastable dynamics provide reasons to suspect that regions reciprocally connected to GC almost certainly contribute to the computation of palatability (and the eventual emission of a consumption decision). LH is one such reciprocally connected region. Here, we began an investigation into the cooperation between LH and GC related to the palatability calculation. Using multi-site *in vivo* electrophysiological recordings and optogenetics in behaving rats we demonstrate functional connectivity between LH and GC for the first time. In experiments utilizing four of the basic tastes that range in palatability, we show optogenetic inhibition of GC-projecting LH terminals for 2.5 seconds in half of our trials reduces the correlation to palatability in laser-on trials exclusively within the palatability epoch (700-1000ms post taste delivery). We go on to recapitulate our Li et al., 2013 finding that there are two types of taste responses in LH with opposing correlations to palatability. Then using dual-site electrophysiology we find change points related to LH palatability-related firing onset couples with ensemble dynamics in GC. In a subset of experiments, electromyography was used to identify taste-reactive behavior and suggest LH ensembles also couple with the onset of behavior. Therefore, we suggest a role for LH-GC cooperation in the palatability calculation and the transition to the decision state and behavioral output.

Disclosures: K. Maigler: None. **D.B. Katz:** None.

Poster

P429. Taste III

Location: Virtual Only

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Program #/Poster #: P429.08

Topic: D.05. Olfaction and Taste

Support: DC007703
DC006666

Title: Illness alters the functional code of gustatory cortex neurons

Authors: *B. T. STONE[1], A. MAHMOOD[2], J.-Y. LIN[1], D. B. KATZ[3];
[2]Neurosci., [3]Dept Psychol, [1]Brandeis Univ., Waltham, MA

Abstract: An animal's reactions to sensory input are influenced by external (e.g. environmental context) and internal (e.g. health-related) variables. These contextual variables shape the perception of a stimulus, thereby guiding decision making. For instance, systemic illness, such as that induced by the intake of mild toxins (e.g. lithium chloride; LiCl), affects an animal's feeding behavior, likely because of modified taste perception: sickness is thought to change taste

palatability, making even normally preferred substances aversive. The specific impact of an ill state can be long-lasting, permanently changing the processing of a stimulus with which subsequent (or even in certain circumstances contemporaneous) illness has been associated. This coupling between malaise and taste makes it reasonable to propose that illness may manifest, at least in part, as network phenomena involving brain regions which carry out behavior- and learning-related taste responses. However, research on the effects of systemic illness on neural correlates of taste processing are lacking. Using extracellular (single-neuron and local field potentials; LFPs) recordings of gustatory cortical responses to passive taste delivery under two conditions (LiCl-induced sickness and healthy), I demonstrate evidence that sickness alters the network state (reflected in changes in 7-12Hz power) at a latency of 12 min post-injection—the same time point at which concomitant illness-related behaviors emerge. Furthermore, in comparison to a healthy state, illness reduces the neural correlates of palatability (indicated via spearman rank correlations and linear discriminant analyses)-a feature which may elucidate the function that an animal's welfare has on the establishment and maintenance of reward valuation and associative effects of stimuli.

Disclosures: B.T. Stone: None. **A. Mahmood:** None. **J. Lin:** None. **D.B. Katz:** None.

Poster

P429. Taste III

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Program #/Poster #: P429.09

Topic: D.05. Olfaction and Taste

Support: NIH Grant 2R01DC007703-16A1
NIH Training Grant 5R90DA033463-10
Brandeis National Committee Lost Angeles Chapter Endowed Fellowship in Neuroscience and Biomedical Science

Title: Cortical Taste Processing Changes With Taste Experience Across Trials and Days

Authors: *D. SVEDBERG, E. BARASH, D. B. KATZ;
Brandeis Univ., Waltham, MA

Abstract: Laboratory rodents are typically raised in highly taste-impoverished environments, and much of the corpus of taste research has operated under the assumption that taste perception is innate—that the first exposure to a taste is processed "normally." Behavioral phenomena such as taste neophobia, however, suggest that taste perception may change as animals grow familiar to taste stimuli, at least on the time scale of days. Here, we investigate whether taste responses in gustatory cortical (GC) ensembles change with familiarization to novel tastes within a single session, and whether any such changes depend on familiarization at longer time scales. We

observe that the normal coding of taste quality and the temporal structure of taste responses evolve across the initial exposures to taste stimuli. This rapid evolution of ensemble activity in GC indicates that experience is required-that taste processing is not inherent. Specifically, our analyses show rapid enhancement of taste-identity coding following the first 5 taste exposures, as well as that the last of 3 normal epochs in the taste response—which is responsible for determining hedonic value of taste (palatability)—is significantly delayed or absent in the first presentations of a novel taste, consistent with recent behavioral analyses of taste neophobia. We then go on to show that home-cage pre-exposure to sucrose eliminates this quick response-evolution to just the pre-exposed taste, but not naive tastes, suggesting that taste-novelty related changes in processing are taste-specific. These experiments add to a growing literature showing that exposure to even simple sensory stimuli can be a “learning” experience, and demonstrate that this learning process occurs across multiple time scales. As such, they reveal the danger of studying taste perception with a "taste-naïve" subject.

Disclosures: D. Svedberg: None. **E. Barash:** None. **D.B. Katz:** None.

Poster

P429. Taste III

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Program #/Poster #: P429.10

Topic: D.05. Olfaction and Taste

Support: NIH Fellowship 1F30DC019523
NIH Grant 5R01DC018227

Title: Cortical plasticity underlying gustatory perceptual learning in behaving mice.

Authors: *J. KOGAN, A. FONTANINI;
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Abstract: To adapt and survive, animals must learn to discriminate partially overlapping sensory stimuli predicting different outcomes. This phenomenon, known as perceptual learning (PL), has been well described in the visual, auditory, somatosensory and olfactory systems. However, very little is known about gustatory PL and the associated patterns of plasticity. Here we present results addressing this significant gap in the literature. The experiments are based on a newly designed taste PL paradigm for mice, which relies on a two alternative forced choice task. The task consists of a taste sampling period at a central spout, a delay period with no access to spouts, and a decision period with access to two lateral spouts. Mice first learn a simple sucrose vs NaCl discrimination, in which sucrose presentation at the central spout is associated with reward at one lateral spout and NaCl with reward at the other. Once they reach criterion, they are tested with a battery of taste mixtures to establish a pre-learning psychometric performance curve. Next, they

are trained to discriminate between increasingly similar pairs of sucrose/NaCl mixtures. After reaching criteria on one pair (>85% correct trials, 3 consecutive days), they advance to the next. The pairs are 75/25 vs 25/75, 65/35 vs 35/65 and 60/40 vs 40/60 (%sucrose/%NaCl). After reaching criteria on the 60/40 vs 40/60 discrimination, mice are tested again with the battery of mixtures to establish a post-learning psychometric curve. Data from n=6 mice show that the post-learning curve has a steeper slope than pre-learning, indicating that the mice are better able to discriminate between the taste mixtures after extensive training. To record neural activity across sessions as mice learn, we use chronic widefield and 2photon calcium imaging. Imaging is conducted in PV-tdTomato mice to record simultaneously from pyramidal and PV+ interneurons. Activity is recorded during all psychometric testing sessions, as well as the first and last sessions of 60/40 vs 40/60 learning. We observe increased numbers of neurons discriminating the 60/40 vs 40/60 mixtures, improved population decoding of mixture identity and increased slope of neurometric curves after learning. These changes are observed both in the sampling and delay periods, consistent with the importance of GC activity during both periods in gustatory PL. These experiments further elucidate the neural basis of PL and overall provide novel information about taste processing in excitatory and inhibitory GC circuits.

Disclosures: J. Kogan: None. **A. Fontanini:** None.

Poster

P517. Cellular Physiology

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P517.01

Topic: E.03. Basal Ganglia

Support: 1ZIAAA000407-19

Title: The synaptic organization of the associative and sensorimotor basal ganglia output

Authors: *G. SITZIA[1], S. BARISELLI[2], D. M. LOVINGER[3];
[1]NIH/ Karolinska Institutet Individual Grad. Partnership, Washington, DC; [2]NIAAA, NIH, Bethesda, MD; [3]Chief, Lab. Integrative Neurosci, Natl. Inst. on Alcohol Abuse and Alcoholism Rockville Office, Rockville, MD

Abstract: Parallel cortico-basal ganglia-thalamo-cortical loops contribute to associative, sensorimotor and emotional behaviors. Glutamatergic inputs from associative and sensorimotor cortices segregate respectively onto the dorsal medial striatum (DMS) and dorsal lateral striatum (DLS). This topographic logic is maintained in the BG output projections to thalamic, brainstem and midbrain areas. The substantia nigra pars reticulata (SNr) is the main BG output in mice. The SNr integrates inputs from striatal 'direct pathway' spiny projection neurons (dSPNs) and 'indirect pathway' inputs from the subthalamic nucleus (STN) and globus pallidus (GPe). The

intrinsic electrophysiological properties, molecular identities and output projections of SNr neurons vary across the medio-lateral axis. Furthermore, medial SNr neurons (^{m}SNr) are innervated by the DMS, and lateral SNr neurons (^{L}SNr) are innervated by the DLS. We hypothesized that this medio-lateral gradient in SNr neuron properties might also underlie differences in the synaptic integration of direct and indirect pathway inputs. We used optogenetic-assisted circuit mapping combined with whole-cell electrophysiology to label SNr neuronal subpopulations as part of associative (DMS-targeted) or sensorimotor (DLS-targeted) BG loops. We found that the direct pathway DMS-^{m}SNr and DLS-^{L}SNr synapses have similar strengths. Further, ^{m}SNr neurons had lower frequency and smaller amplitude of spontaneous GABAergic IPSCs compared to ^{L}SNr neurons. Next, we studied indirect pathway STN-^{m}SNr and STN-^{L}SNr synapses. We found that the STN targets ^{m}SNr and ^{L}SNr neurons with similar probability. Surprisingly, STN-^{m}SNr synapses were comparatively weaker than STN-^{l}SNr synapses. These results reveal a synaptic basis for a differential control of associative and sensorimotor basal ganglia output neurons by the indirect pathway.

Disclosures: G. Sitzia: None. **S. Bariselli:** None. **D.M. Lovinger:** None.

Poster

P517. Cellular Physiology

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Program #/Poster #: P517.02

Topic: E.03. Basal Ganglia

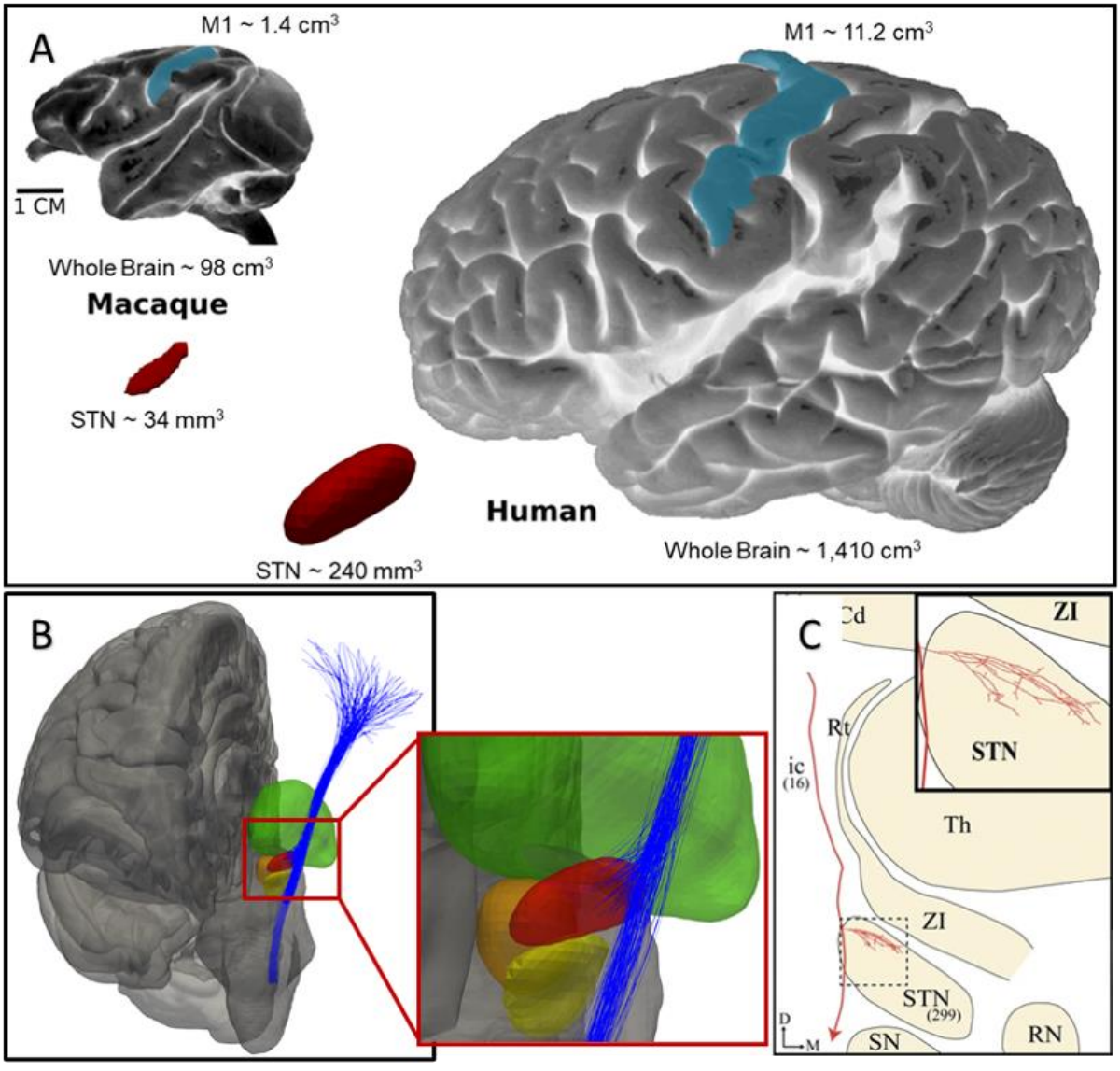
Support: NIH R01 NS105690
NIH R01 NS086100

Title: Deep brain stimulation of hyperdirect axon collaterals

Authors: *C. S. BINGHAM, C. C. MCINTYRE;
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Abstract: Various mechanisms of subthalamic deep brain stimulation have been proposed over the last quarter century, many recent iterations of which focus on the tendency for extracellular electrical stimulation to preferentially activate large axons of prominent basal ganglia white matter pathways. Despite numerous empirical studies of subthalamic deep brain stimulation and anatomical descriptions of the basal ganglia, an adequate understanding of the role that important pathways, such as the hyperdirect pathway, play in mechanisms of DBS remains elusive. Recent revelations in basal ganglia white matter anatomy and advances in computing have made model-based examination of the motor hyperdirect pathway under STN-DBS possible. As such, this study presents a new model of the human motor hyperdirect pathway, anatomically contextualized within the CIT168 head, and computes estimates of pathway behavior under

cathodal, anodal, and bipolar current-controlled stimulation. The chief results show that while very large diameter fibers are likely to be directly recruited in the internal capsule at very low amplitudes, a realistic mixture of fiber diameters results in a strong spatial and temporal smearing both within the STN and in the primary motor cortex. Anodal stimulation increases activation thresholds but also increases the likelihood of activation within the terminal collaterals over the corticofugal fibers, resulting in longer latencies at the cortex. Bipolar stimulation dramatically increases activation thresholds but provides for much greater selectivity for collaterals over corticofugal fibers in the internal capsule. Lastly, relative to anatomically detailed collateral arbors, simple and unbranched collaterals only slightly underpredict both recruitment thresholds and the likelihood of preferential collateral activation with large diameter fibers at low-range amplitudes, but the ability to describe spatiotemporal patterns within the terminal region is greatly enhanced when anatomical realism is properly represented.



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Poster

P517. Cellular Physiology

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Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P517.03

Topic: E.03. Basal Ganglia

Support: NIH R01 DA041705
CRC1080

Title: Potentiation of fast Kv4.3 currents after dopamine neuron-specific KChIP4a knockout strengthens inhibitory signaling in silico

Authors: *C. J. KNOWLTON[1], K. M. COSTA[2,3], C. C. CANAVIER[1], J. ROEPER[3]; [1]LSU Health, New Orleans, New Orleans, LA; [2]NIH, Baltimore, MD; [3]Goethe University, Frankfurt, Franfurt, Germany

Abstract: Inhibitory inputs to dopamine neurons (DA) in the midbrain are believed to drive extinction learning by evoking pauses in firing in the absence of expected reward. Within the VTA, subpopulations are characterized by dramatically different responses to hyperpolarization applied in vitro, suggesting that these intrinsic subthreshold differences may play a role in integration of inhibitory inputs. DA neurons that project to the core/medial shell are characterized by both a minimal sag potential and a prolonged (1+ s) ramp-like delay after the offset of a hyperpolarizing step, whereas lateral shell and dorsal striatum-projecting cells are characterized by both significant sag potentials and a short (< 500 ms) ramp-like delay followed by a rebound “burst” or phasic increase in firing rate. In vitro experiments have shown that the longer, ramp-like delays are due to the slower inactivation of Kv4.3-mediated K channels. We have recently discovered that this slower inactivation is driven by selective expression of the KChIP4a slice variant, an auxiliary subunit of the Kv4.3 channel complex. Somewhat counterintuitively, suppression of the slower KChIP4a subunit in a dopamine neuron-specific knockout was found to significantly increase the rate of extinction learning in mice, which implies enhanced negative reward prediction error (RPE) signals. Based on the biophysical characteristics of heterologously expressed KChiP4a-Kv4.3 channels, we integrated these channels into our recently developed single compartment in silico models of core-projecting ("atypical") VTA DA neurons. In addition, we simulated an in vivo-like balanced excitation-inhibition (E-I) state and applied phasic bouts of increased inhibition to simulate repeated

negative RPE trials. The in vivo simulations showed that the removal of the KChIP4a splice variant - resulting in higher conductance of faster inactivating A-type K currents, significantly increased the pauses that follow phasic inhibition, thereby providing a candidate mechanism for the enhanced extinction learning in the dopamine-selective KChIP4a knockout mice. Currently, we are expanding these cell models to include realistic morphologies as well as biophysical properties of native A-type K channels from wildtype and dopamine-specific Kchip4a-KO mice.

Disclosures: C.J. Knowlton: None. **K.M. Costa:** None. **C.C. Canavier:** None. **J. Roeper:** None.

Poster

P517. Cellular Physiology

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P517.04

Topic: E.03. Basal Ganglia

Title: Regulation of striatal circuit formation by the nuclear-localized protein Zswim6

Authors: *N. T. HENDERSON[1], K. CHOI[1], D. TISCHFIELD[1], S. A. ANDERSON[2], M. V. FUCCILLO[1];
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Abstract: The striatum integrates synaptic inputs from multiple brain regions and is crucial for many motor and cognitive processes. Despite these central functions and its association with brain disease, relatively little is known about the genetic and molecular mechanisms governing striatal circuit development and maintenance. Zswim6 (Zs6) is a striatally expressed gene that is implicated in Schizophrenia, intellectual disability, and educational attainment. The function of Zs6 is unknown, but the presence of SWIM and sin3-like domains suggest a role in gene regulation. Previous work in Zs6 constitutive knockout (KO) mice revealed defects in dendrite and synaptic morphology in striatal spiny projection neurons (SPNs), and behavioral deficits consistent with striatal dysfunction. To further assess the function of Zs6 in the development of striatal circuits, we utilized conditional Zs6 KO (cKO) mice together with acute slice synaptic physiology, morphological analyses and cell culture. Conditional deletion of Zs6 in SPNs did not affect cell morphology, but impacted specific aspects of synaptic function. Zs6 deletion in either direct pathway or indirect pathway SPNs (dSPNs or iSPNs, respectively) caused an increase in paired-pulse ratio (PPR), suggesting decreased synaptic release probability. These findings suggest that Zs6 plays a role in regulating synaptic function in SPNs. Given the presence of SWIM and sin3-like domains, which are found commonly found in chromatin remodeling proteins, we tested the hypothesis that Zs6 functions as a transcriptional regulator. Utilizing primary neuron cultures, we expressed FLAG-tagged Zs6 to determine its subcellular

localization and biochemical interactions. In line with a role for Zs6 in gene regulation, we found that Zs6 localized almost exclusively to the nucleus in primary neurons. Additionally, Zs6 biochemically associated with chromatin when expressed in HEK293T cells. Together, our findings suggest that Zs6 may influence the development or maintenance of synaptic inputs to SPNs via transcriptional regulation. Future experiments will entail direct assessment of this hypothesis using RNA-seq and ChIP-seq to determine the role of Zs6 in transcriptional regulation, in addition to further electrophysiological analysis of the effects of Zs6 deletion at the synaptic and circuit levels. Together, these experiments will provide insights into the mechanisms by which Zs6 regulates striatal circuit function.

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Poster

P517. Cellular Physiology

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Program #/Poster #: P517.05

Topic: E.03. Basal Ganglia

Support: Brain and Behavior Research Foundation NARSAD Young Investigator Award
McNair Scholars Program
Murchison and Biology Summer Research Fellowship

Title: Functional dissection of ipsilateral versus contralateral projections from the pedunculopontine tegmental nucleus to dopaminergic neurons

Authors: *D. PENNY[1], C. HOFFMAN[1], I. KHETARPAL[1], M. NEELALA[1], E. FARRELL[1], M. HORN[1], J. CLARK[2], L. MUZYKA[1], J. VALADEZ[1], C. WILLIAMS[2], G. M. BEAUDOIN, III[3]; [1]Biol. and Neurosci., [2]Biol. and Chem., [3]Biol., Trinity Univ., San Antonio, TX

Abstract: Dopamine plays an important role in communication between neurons, notably in the nigrostriatal dopaminergic pathway. This pathway is involved in motivation and reward-seeking behavior and is activated by stimulatory inputs. Our research is studying the synapses between the midbrain regions pedunculopontine tegmental nucleus (PPN) and substantia nigra pars compacta (SNc). The PPN is composed of three main phenotypes of neurons, cholinergic, GABAergic, and glutamatergic. Previous research has shown that PPN fibers contain more projections to the SNc and exert stronger activity in the nigrostriatal dopaminergic pathway. While many brain nuclei confine their outputs either ipsilaterally or contralaterally, the PPN appears to project to both. These two projections from PPN have not been extensively studied electrophysiologically due to a lack of tools. To further understand the strength of this synapse, unilateral and bilateral injections in the PPN are performed to determine ipsilateral and

contralateral communication from PPN to SNc. To test the strength of this synapse, we are utilizing optogenetics, a method that allows us to selectively stimulate PPN neurons and record responses to SNc dopamine neurons. To determine if SNc dopaminergic neurons receive ipsilateral and contralateral input from the PPN, we are using opsins activated by two different wavelengths, channelrhodopsin-2 and ChrimsonR. We have optimized a virus encoding the red-shifted opsin, ChrimsonR, tagged with green fluorescent protein for these experiments. To better characterize these PPN projections, we are investigating the neurotransmitter type and receptor types from each projection using pharmacology to block specific classes of receptors. Injection site images of both bright-field, yellow fluorescent protein (YFP), and red fluorescent protein (RFP) were obtained to ensure the injection sites were properly hit. Our results, obtained through immunohistochemistry and confocal microscopy, confirm previous studies demonstrating PPN projects ipsilaterally and contralaterally to the SNc and subthalamic nucleus (STN). These results will further delineate the neural circuit between PPN and SNc.

Disclosures: D. Penny: None. **C. Hoffman:** None. **I. Khetarpal:** None. **M. Neelala:** None. **E. Farrell:** None. **M. Horn:** None. **J. Clark:** None. **L. Muzyka:** None. **J. Valadez:** None. **C. Williams:** None. **G.M. Beaudoin:** None.

Poster

P530. Reaching Control: Motor Learning: Human Neurophysiology

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Program #/Poster #: P530.01

Topic: E.04. Voluntary Movements

Support: ANPCyT, FONCyT: PICT 2015-0844
MINDEF, PIDDEF 2014/2017#17
ANPCyT, FONCyT: PICT-2018-01150

Title: Visuomotor adaptation promotes spindle clustering and fast spindle-SO coupling locally, over the contralateral motor network

Authors: *A. SOLANO[1], L. A. RIQUELME[1], D. PEREZ-CHADA[2], V. DELLA-MAGGIORE[1];
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Abstract: The last decade has seen remarkable progress in the identification of the neural signatures of sleep-dependent consolidation. Yet, most advances have emerged from the field of declarative memory, possibly due to the substantial evidence suggesting that only hippocampal dependent learning benefit from sleep. Overnight reactivation of hippocampal memories appears to depend on the precise synchrony between slow oscillations (SO) and sleep spindles

(Muehlroth et al., 2019). Here, we sought to examine whether this coupling is also critical for the consolidation of human motor memories. With the objective of identifying the neural signatures supporting sleep stabilization of visuomotor adaptation (VMA) memories in humans, a set of subjects (n = 10) was exposed to a VMA session and a control (CTL) session without visual perturbation. Subjects went to bed 10 min after each training session, and EEG recordings were obtained throughout a whole night of sleep. We found that VMA increased the overall density of fast (≥12 Hz) but not slow (<12Hz) sleep spindles during NREM3 ($\chi^2(1)=12.97$; $p<0.001$). This modulation was manifested locally, over the hemisphere contralateral to the trained hand. Motor learning also increased the proportion of spindles clustered into trains (inter-spindle intervals <= 6 secs), a phenomenon that has recently been linked to memory reactivation (Antony et al., 2018; Boutin & Doyon, 2020). This effect was observed during NREM3 only, over the contralateral hemisphere to the trained hand ($\chi^2(1)=6.63$; $p=0.01$), and the inter-hemispheric modulation correlated significantly with memory retention overnight ($R=0.74$, $p=0.02$). To explore the potential relevance of spindle clustering in sleep-dependent plasticity (Antony et al., 2019), we quantified the impact of VMA on spindle duration and amplitude. We found that motor learning increased these intrinsic parameters (RM-correlation; Duration: $Rrm = 0.75$, $p < 0.001$; Amplitude: $Rrm = 0.76$, $p < 0.001$), a finding consistent with the potential role of spindles as a window of opportunity for memory reactivation and plasticty (Klinzing et al., 2019; Ngo et al., 2020). Finally, although VMA did not affect the density of SOs ($t(9)=-1.07$; $p>0.05$), it substantially enhanced the number of fast spindles locked to the active phase of SOs ($\chi^2(1)=5.82$, $p=0.02$). The fact that only coupled spindles of the contralateral hemisphere predicted long-term memory overnight, points to the spindle-SO association as a fundamental signature of motor memory consolidation. Our work provides evidence in favor of a common mechanism at the basis of the stabilization of declarative and non-declarative memories.

Disclosures: A. Solano: None. **L.A. Riquelme:** None. **D. Perez-Chada:** None. **V. Della-Maggiore:** None.

Poster

P530. Reaching Control: Motor Learning: Human Neurophysiology

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Program #/Poster #: P530.02

Topic: E.04. Voluntary Movements

Support: National Agency for the Promotion of Science and Technology (ANPCyT) Grant PICT-2015-0488
Grant from the Quebec Bioimaging Network (QBIN, Canada)
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Title: Unveiling the contribution of the hippocampus to motor learning

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Abstract: The hippocampus has been long associated with the codification of declarative memories. Recently, however, a few studies carried out in human and non-human animals have linked it to the consolidation of non-declarative memories (Dohring et al., 2017; Sawangjit et al., 2018; Schapiro et al., 2018). For example, we have shown that improvements in performance observed during the quiet rest periods of learning a novel motor sequence (MSL) are associated with an increment in hippocampal activity (Jacobacci et al., 2020), and microstructural plasticity (Sagi et. al, 2012) 30 min post learning, reflected as a reduction in mean diffusivity (MD). Some current theories may explain the role of the hippocampus in the encoding of both declarative and non-declarative memories. Buzsaki and Tingley (2018) proposed that it may act as a sequence generator, connecting encoded items of different modalities in space/time. Another -non exclusive- view posits that it may be involved in associating nearby stimuli in a sequence explicitly, during early stages of learning (Krakauer et al., 2019). Here, we carried out two MRI studies to test these hypotheses. To address the former, subjects (n = 18) learned a visuomotor adaptation task (VMA) that involves no sequencing. Like MSL, learning this task depends on an initial explicit component (Taylor et al., 2014). Thus, to address the second hypothesis, a different set of subjects (n = 20) learned an implicit version of the VMA task (IVMA; Hyosub et al., 2019). In both experiments, 3 Diffusion Weighted Image acquisitions were obtained before, 30 minutes and 24 hours after training, and Diffusion Tensor Imaging was applied to quantify microstructural changes based on MD. As in our previous study (Jacobacci et. al., 2020), we found that training on either VMA or IVMA decreased MD in the left hippocampus 30 minutes post-learning (SwE, $p<0.05$ FWE-corrected), and that this reduction predicted overnight memory retention. Yet, microstructural changes induced by VMA returned to baseline values by 24 hs, whereas those induced by IVMA were longer lasting (SwE, $p<0.016$ FWE-corrected). This difference may stem from the supremacy of the implicit component leading to stronger retention (Scott et al., 2020) and plasticity. Our results do not support a role of the hippocampus in sequence generation or explicit processing. We instead propose that, during learning, the hippocampus may act as a short-term memory buffer transiently reactivating information necessary to predict actions/items in upcoming iterations, regardless of data structure -sequential or not-, the learned material -words or actions-, and the nature of the task -implicit or explicit-.

Disclosures: G. Griffa: None. **F. Jacobacci:** None. **V. Della Maggiore:** None.

Poster

P530. Reaching Control: Motor Learning: Human Neurophysiology

Location: Virtual Only

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Program #/Poster #: P530.03

Topic: E.04. Voluntary Movements

Support: Grant from the National Scientific and Technical Research Council, Argentina (CONICET)
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grant from the Quebec Bioimaging Network (Canada)

Title: Motor sequence learning rapidly drives a transient state of increased network segregation and decreased hippocampal-somatomotor interactions in resting-state connectivity

Authors: *A. DELEGLISE[1], P. DONELLY-KEHOE[2], A. YEFFAL[1], F. JACOBACCI[1], J. ARMONY[3], J. JOVICICH[4], E. AMARO JR[5], J. DOYON[6], V. M. DELLA MAGGIORE[1]; [1]IFIBIO Houssay, Sch. of Medicine, UBA, Buenos Aires, Argentina; [2]Dept. of Res. and Innovation, Kozaca SA, Rosario, Argentina; [3]Douglas Mental Hlth. Res. Institute, McGill Univ., Montreal, QC, Canada; [4]Ctr. for Mind/Brain Sciences, Univ. of Trento, Rovereto, 38068, Trento, Italy; [5]Plataforma de Imagens na Sala de Autopsia (PISA), Inst. de Radiologia, Facultade de Medicina, Univ. de Sao Paulo, Sao Paulo, Brazil; [6]McConnell Brain Imaging Ctr., Montreal, QC, Canada

Abstract: Motor sequence learning (MSL) refers to the acquisition of a new sequential motor program. We have recently shown that improvements in performance during the quiet rest periods interspersed with practice of a novel sequence (micro-offline gains, Bonstrup et. al., 2019) are associated with increased activity in the hippocampus during early learning, and microstructural changes in mean diffusivity 30 min post-training. In the present work we aimed to characterize how learning-related changes in the hippocampus impacted on its cortical connectivity. To this aim we collected resting-state functional magnetic resonance imaging data from 21 subjects before, 30 min and 24 h after training on a five item (MSL) or an active control task involving no learning. To identify the learning-specific effects on network connectivity and topology, we performed a network analysis using three different approaches: a global analysis, a connectome-wide analysis comprising edge- and node-level analyses, and a multivariate analysis with machine learning techniques. At a global level, we found that network modularity increased 30 minutes after performing the MSL task (permutation test, $p = 0.005$), and this increase was followed by a return of modularity to its baseline state 24 hours post learning. Interestingly, this increase in modularity was associated with the amount of total learning during MSL ($r= 0.48, p<0.05$). At the edge-level, MSL drove connectivity changes in a network mainly composed of connections between cortical and subcortical regions. Importantly, the anterior portion of the left hippocampus yielded the highest degree in this network, and its connections were all restricted to a group of bilateral somatomotor network regions. At the nodal level, we found a decrease in the intermodular connectivity of a right hemisphere post-central sulcus somatomotor region 30 minutes after MSL training (Wilcoxon Signed Rank test $p = 0.014$), which returned to baseline levels at 24 hours. Finally, a feature selection and supervised classification showed that four somatomotor-hippocampal connections serve to discriminate an MSL pre-learning network state from a post-learning network state with an accuracy of 80.95%; again, the anterior region of the left hippocampus was the region with higher degree in this connectivity pattern. Altogether, our results deepen in the rapid functional network reorganization induced by MSL, highlighting

the critical role of hippocampal-somatomotor connections, the increase in network segregation, and the decrease in between-modules connectivity of a post-central sulcus somatomotor region.

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Poster

P530. Reaching Control: Motor Learning: Human Neurophysiology

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Program #/Poster #: P530.04

Topic: E.04. Voluntary Movements

Support: Penn State Social Science Research Institute (SSRI) Grant 150000004354

Title: Anodal (excitatory) HD-tDCS dissociates a lateralized role of the posterior parietal cortex in both early and late phases of learning

Authors: *B. DEXHEIMER[1], C. W. SANDBERG[2], K. SELBY[1], S. JAYASINGHE[3], S. S. KANTAK[4], R. L. SAINBURG[1,3];
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Abstract: The functional neuroanatomy underlying motor learning remains poorly understood, and this research is further complicated by differing neural processes that may contribute to the many aspects of motor learning, including different task types (adaptation vs. skill learning), different types of learning (implicit vs. explicit), and different phases of learning (early vs. late). We have previously studied motor learning through the investigation of adaptation to visuomotor rotation (VMR). This research has demonstrated that focal lesions to the left, but not right, posterior parietal cortex (PPC) prevent adaptation to VMR, regardless of the arm used for the task. While these findings suggest a lateralized role of the left PPC for adaptation, it's contribution to fast and/or slow components of learning are not well understood. Kumar et al. (2020) recently utilized high-definition transcranial direct current stimulation (HD-tDCS) to show that left, but not right, PPC inhibition disrupts adaptation to VMR, primarily in the late phase of learning. This suggests that excitatory HD-tDCS to the left PPC may enhance VMR adaptation, specifically slow learning processes, thus demonstrating potential as an effective technique within stroke rehabilitation. However, it is also plausible that left PPC excitation has no impact on motor learning. To investigate this, we tested participants on a VMR task in a 2D virtual environment, while applying either anodal HD-tDCS or sham stimulation to their left PPC. Participants performed baseline reaches to eight virtual targets, followed by the introduction of a 30-degree VMR, then a period of de-adaptation (VMR removed). Performance

was quantified by initial direction error, reflecting feedforward mechanisms, along with deviation from linearity and final position error, which reflect both feedforward and feedback control mechanisms.Our preliminary results (12 participants) indicate that anodal HD-tDCS leads to lower initial direction error overall ($p = .028$), and lower error variability ($p = .005$). In terms of deviation from linearity, there was no difference between the groups in the early phase of learning ($p > .999$), but a significant difference in the late phase ($p < .0001$), with the HD-tDCS group performing straighter reaches. Interestingly, for final position error, there was an overall non-significant trend towards higher error for the HD-tDCS group ($p = .088$). These findings indicate a potentially lateralized role of the left PPC in adaptation to VMR across both the early (primarily fast) and late (primarily slow) phases of learning.

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Poster

P530. Reaching Control: Motor Learning: Human Neurophysiology

Location: Virtual Only

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Program #/Poster #: P530.05

Topic: E.04. Voluntary Movements

Support: NSF CBET Grant 1943712
AHA Scientist Development Grant 17SDG33690002

Title: Changes in resting state functional connectivity associated with dynamic adaptation of the wrist and alternate motor control strategies

Authors: *A. FARRENS[1], F. SERGI[2];
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Abstract: Dynamic adaptation is an error-driven process of adjusting our planned motor actions to changes in task dynamics. Adapted motor plans are consolidated into motor memories that contribute to better performance on re-exposure to the same dynamic condition. In parallel, dynamic perturbations can be compensated for by alternate motor control processes, such as co-contraction, that contribute to error reduction. Whether these motor control strategies share the same neural resources for memory formation is unclear. To address this, we used an fMRI-compatible wrist robot, the MR-SoftWrist, to identify neural processes specific to dynamic adaptation and subsequent motor memory formation. Using the MR-SoftWrist, 30 healthy young adults performed a wrist pointing task in a zero force condition (control condition), and in a lateral force condition (dynamic condition) during fMRI to localize task-related brain networks. Resting state fMRI scans were acquired immediately pre- and post-performance of both tasks to quantify changes in resting state functional connectivity (rsFC) within these networks. We

assessed behavioral retention of training 24 hours later. A variance decomposition analysis was used to isolate behavior associated with adaptation versus alternate error reduction strategies. Immediately after the lateral force task, rsFC significantly changed in two brain networks: rsFC increased within the cortico-thalamic-cerebellar (CTC) network of the trained wrist and decreased interhemispherically within the cortical sensorimotor networks. Linear regression analysis returned significant associations between rsFC measured within the sensorimotor network and day 1 measures of error reduction, while changes within the CTC network were associated with behavioral measures of adaptation and retention, indicative of memory formation. Variance decomposition analysis indicated that increases within the CTC network were associated with adaptation, while decreases in interhemispheric sensorimotor rsFC were associated with alternate error reduction processes.

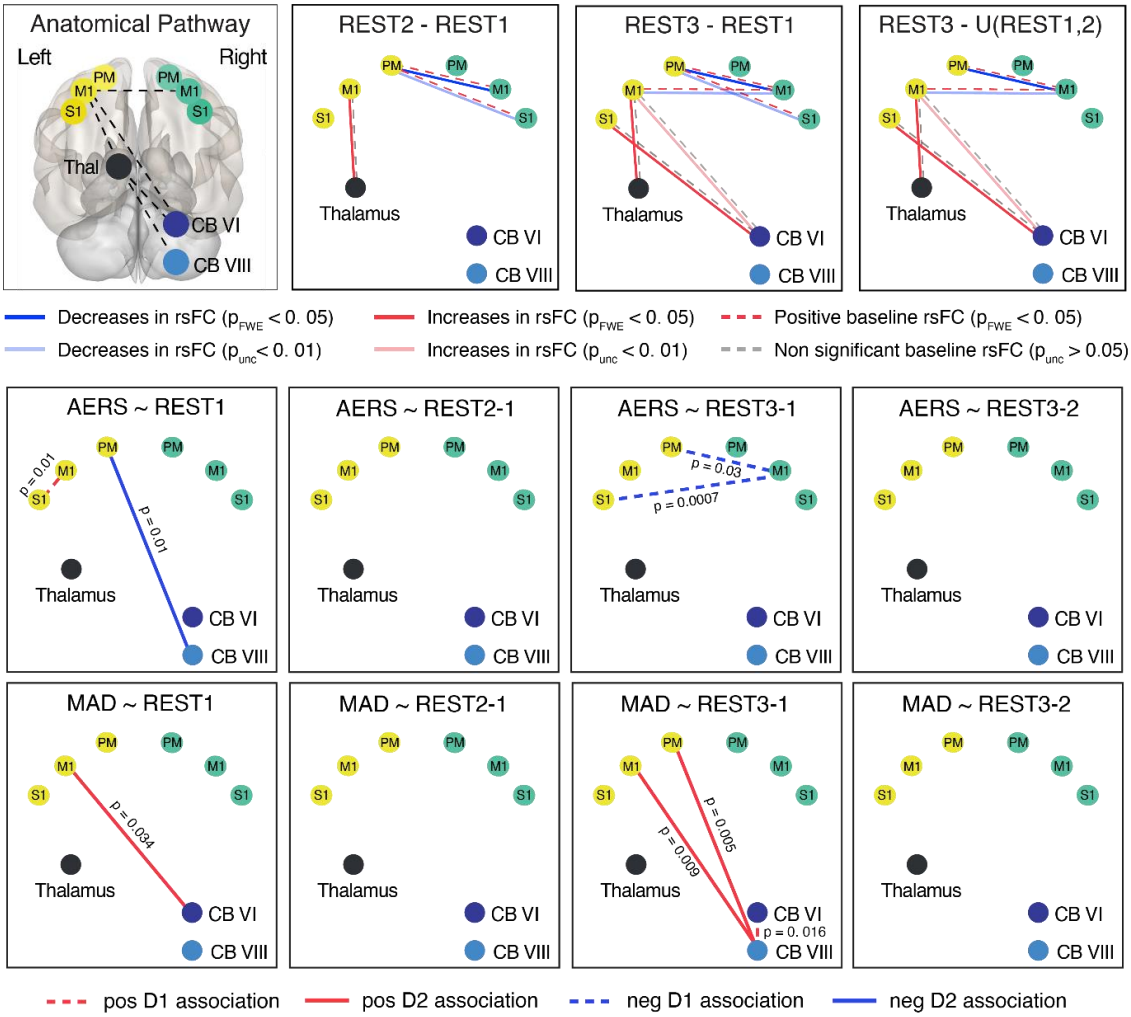


Figure 1. Top: Changes in rsFC from baseline (REST 1) immediately following the motor task performed in the zero-force condition (REST 2), and the dynamic perturbation task performed in the lateral force condition (REST 3). Mixed model analysis showed significant decreases in interhemispheric rsFC within the sensorimotor network following both tasks, and significant increases in the cortico-thalamic-cerebellar network following the dynamic perturbation task. **Bottom**: Results of a linear regression analysis between changes in rsFC to behavioral metrics of alternate error reduction strategies (AERS) or adaptation specific processes (MAD). RsFC measured within the sensorimotor network was associated with alternate motor control strategies contributing to task performance on day 1, while increases within the cortico-thalamic-cerebellar network were associated with adaptation specific motor memory formation.

Disclosures: A. Farrens: None. **F. Sergi:** None.

Poster

P530. Reaching Control: Motor Learning: Human Neurophysiology

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Topic: E.04. Voluntary Movements

Support: NIH Grant NS116883

Title: Interference underlies the attenuation observed during relearning of a sensorimotor perturbation

Authors: *G. AVRAHAM, M. MALAVIYA, R. IVRY;
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Abstract: Savings describes the benefits in performance resulting from the recall of a previously learned behavior. In sensorimotor adaptation, savings is tested by presenting the same perturbation over two learning blocks separated by washout. Savings, manifest as faster relearning, has been attributed to a recall of an explicit strategy (Haith et al. 2015, Morehead et al. 2015). We have recently shown that implicit adaptation does not contribute to savings, and is actually attenuated upon relearning (Avraham et al. 2021). To understand this attenuation, it is important to note that, due to the removal of the perturbation, participants are exposed to an opposite error during washout. A persistent trace of this experience may produce interference during relearning. To test this hypothesis, we used a visuomotor task that isolates implicit adaptation. While reaching to a target, the cursor followed an invariant path with an angular offset from the target. Despite instructions to ignore the cursor, participants show implicit adaptation to this clamped feedback, with the hand path shifting in a direction away from the target (and cursor). For one group (Active Washout), washout was induced by an initial exposure to an opposite clamped feedback followed by an extended period of veridical feedback. For another group (Passive Washout), all feedback was eliminated during washout; here participants exhibited a gradual, often incomplete decay back towards baseline. The Active group showed marked attenuation, with a ~30% decrease in aftereffect in the second learning block. In contrast, the Passive group did not show any sign of attenuation. Importantly, the absence of attenuation was not due to incomplete washout: There was no correlation between the hand angle measured during late washout and a measure of attenuation ($r=0.03$, $p=0.831$). These results suggest that attenuation requires experience with an error signal inconsistent with that experienced during learning. We next tested if prolonged exposure to veridical feedback prior to initial learning would produce attenuation. A new group (Prolonged Baseline) received veridical feedback for same number of trials as an Active group experienced in the initial learning and washout blocks. The learning of the Prolonged group was not attenuated; it was comparable to the initial learning

of the Active group. Thus, veridical feedback, even in the same experimental context as subsequent perturbed feedback, is not a source of interference. Overall, the results support the hypothesis that implicit adaptation is subject to interference effects between different errors experienced in a changing environment.

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Poster

P530. Reaching Control: Motor Learning: Human Neurophysiology

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Program #/Poster #: P530.07

Topic: E.04. Voluntary Movements

Title: Reinforcement feedback changes Beta and Gamma neural oscillations during visuomotor learning

Authors: *C. HILL[1], D. E. WADDELL[2], A. J. DEL ARCO[3,4];
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Abstract: Reinforcement feedback (i.e. reward and punishment) have dissociable effects on the learning of visuomotor adaptation tasks and differently affect the associated EEG activity (i.e. event-related potentials). Beta (β) and Gamma (γ) neural oscillations contribute to task learning and are sensitive to reinforcement feedback. However, it is currently unclear whether reinforcement feedback changes Beta and Gamma oscillatory activity during motor learning. . Participants were randomly placed into one of three groups [Reward (n=14), Punishment (n=14), Control (n=14)] and performed 280 trials of a visuomotor rotation task [Baseline (80 trials), Adaptation (200 trials)] with a Wacom pen and tablet. Adaptation (learning phase), featured an incongruent position between the cursor and the target, with the cursor trajectory rotated 30-degrees counterclockwise to the target, requiring participants to adapt their movement to hit the target. During Adaptation, after each trial, feedback was provided based on error magnitude in the form of a positive number (Reward), negative number (Punishment) or two vertical lines (Control). Positive and negative numbers represented a monetary gain and loss, respectively. EEG was recorded in during the Adaptation condition from 28 electrodes to measure spectral power of β (13-30Hz) and γ (30-50Hz) frequency bands after feedback presentation. Learning was quantified by calculating the average change in angular error (ΔΘ) trial to trial during Adaptation. We found that all feedback groups displayed similar reductions in error trial to trial throughout the learning phase ($F(2,39)=1.21$, $p=0.31$, ANOVA), suggesting reinforcement feedback does not alter task learning [Reward= -0.078ΔΘ, Punishment= -0.096ΔΘ, Control= -0.123ΔΘ]. Reward, but not Punishment, demonstrated an increased spectral power in β

(F(2,36)=3.87, p=0.03, ANOVA) compared to Control during Adaptation. Moreover, both Reward and Punishment demonstrated a decreased spectral power in γ (F(2,36)=5.19, p=0.011, ANOVA) compared to Control (p=0.014 and p=0.033 respectively). These results suggest that cortical β and γ oscillations reflect the saliency of reinforcement feedback and may operate as a marker of different error monitoring processes utilized during the task.

Disclosures: C. Hill: None. **D.E. Waddell:** None. **A.J. Del arco:** None.

Poster

P530. Reaching Control: Motor Learning: Human Neurophysiology

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Program #/Poster #: P530.08

Topic: E.04. Voluntary Movements

Support: The Foundation for Neurofeedback & Neuromodulation Research Mini-Grant #104-2020

Title: Relating Resting-State EEG Frontoparietal Coherence with Visuospatial and Motor Skill Performance

Authors: P. WANG[1], K. R. LOHSE[3], A. PATHANIA[4], N. K. HAIKALIS[1], S. Y. SCHAEFER[2]; [1]Biomed. Engin., [2]SBHSE, Arizona State Univ., Tempe, AZ; [3]Physical Therapy, Washington Univ. in St. Louis, St. Louis, MO; [4]Hlth. and Kinesiology, Univ. of Utah, Salt Lake City, UT

Abstract: Visuospatial ability may explain individual variabilities in the extent of motor skill learning. Recent research suggested that better visuospatial scores correlate with more retention. Neuroimaging evidence suggests that the frontoparietal structures underlie both visuospatial performance and visuomotor learning. Furthermore, neuropsychological findings suggest that right frontoparietal networks specifically may be critical for this relationship, as many visuospatial processes are specialized to the right parietal cortex. Thus, this proof-of-concept study aims to test whether frontoparietal functional connectivity at rest, measured by resting-state EEG coherence, is related to both visuospatial performance and early motor skill acquisition. A retrospective dataset of 21 participants was analyzed, with 2-min eyes-closed resting state EEG, Visuospatial/Constructional Index score from the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) and five trials of motor practice on a functional motor task. Right frontoparietal coherence in the alpha band (8-12Hz) was computed with imaginary coherence (IC) between electrodes F4 and P4. ICs from the left (F3-P3) and midline (Fz-Pz) electrodes were also included as negative controls. Results indicated that F4-P4 alpha IC was highly correlated with the RBANS Visuospatial Constructional Index (r = 0.55, p = .035), while left and midline alpha ICs were not (all ps > .140). This study extends previous structural findings and indicated that frontoparietal functional connectivity, especially in the right

hemisphere, may underlie visuospatial function. However, this study did not find a correlation between right frontoparietal alpha IC with motor skill acquisition ($p = .474$), measured as within-session rate of improvement. This null finding is likely due to the limited dose of motor practice (only 5 trials) in the retrospective dataset, which was not inherently designed to investigate motor skill acquisition per se. Further studies are needed to use a larger training dose to accurately evaluate motor skill acquisition and its relationship with right frontoparietal coherence. However, this study supports that right frontoparietal IC is positively related with visuospatial function. This finding has implications for developing right frontoparietal alpha IC-based neurofeedback applications for cognitive training in and of itself, or to benefit slow- or non-learners in motor rehabilitation.

Disclosures: P. Wang: None. **K.R. Lohse:** None. **A. Pathania:** None. **N.K. Haikalis:** None. **S.Y. Schaefer:** None.

Poster

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Location: Virtual Only

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Program #/Poster #: P530.09

Topic: E.04. Voluntary Movements

Support: NSERC
CIHR

Title: Distinct patterns of cortical manifold expansion and contraction underlie human sensorimotor adaptation

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Abstract: Previous studies have implicated a widely distributed network of cortical regions in sensorimotor adaptation that span motor, sensory, and cognitive networks. This work shows that the recruitment of these regions can vary throughout different stages of adaptation. However, the nature of interactions between these regions during learning, particularly within the context of cortical functional organization, remain poorly understood. To probe the network processes that underlie sensorimotor learning, we capitalized on recent connectivity gradient approaches that examine the low-dimensional structure of brain-wide patterns of connectivity. In two identical fMRI sessions, scans were collected while subjects (N = 32) adapted to an instantaneous 45-degree rotation applied to the cursor, following Baseline trials, during an 8-target center-out

movement task performed with the right hand. We characterized cortical functional connectivity patterns by a low-dimensional manifold, in which the top three principal components consisted of sensorimotor, visual, and frontoparietal gradients. Adaptation-related changes in functional integration and segregation were identified by manifold contraction and expansion, respectively, during the Early and Late adaptation phases. Relative to Baseline, Early adaptation was marked by manifold contraction in several motor (e.g., dorsal premotor cortex), parietal (e.g., intraparietal sulcus), and higher-order association (e.g., posterior cingulate cortex) regions in the contralateral (left) hemisphere. These regions also remained contracted during Late adaptation—wherein learning was largely complete—but were additionally accompanied by expansion of bilateral visual areas along the manifold (e.g., areas V3 and V4, lateral occipital cortex). We further demonstrate that the extent of manifold contraction of sensorimotor regions is related to individual differences in learning, such that greater contraction of sensorimotor regions is associated with lower overall error during Early adaptation. Together, these findings suggest that sensorimotor adaptation is characterized by the increased integration of contralateral sensorimotor regions within the cortical manifold, as well as the later segregation of visual areas from this same manifold, at a time when the contribution of visual feedback to performance is likely diminished.

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Poster

P530. Reaching Control: Motor Learning: Human Neurophysiology

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Topic: E.04. Voluntary Movements

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Title: The effect of multiple concussions on rule-based and basic visuomotor performance in humans.

Authors: S. ENTEZAMI[1], M. DALECKI[2], N. SMEHA[1], J. A. BROWN[3], A. CAVALIERE[1], J. M. HURTUBISE[4], A. K. MACPHERSON[1], *L. E. SERGIO[1];
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Abstract: Sustaining a concussion can affect many aspects of daily life, including basic and complex motor performance. While long-term effects of any concussion are increasingly documented, less is known about the effect of multiple concussions on skilled motor behavior. Recent rodent models of repeated concussion have noted increases in reactive oxidative species production, excessive excitatory amino acids and nitric oxide, and intracellular Ca2+ accumulation, all leading to Blood-Brain Barrier dysfunction and consequent pathology development. While our previous work in humans has consistently shown a decline in rule-based visuomotor performance (i.e., cognitive-motor integration,CMI) in those with a history of concussion (vs. no history), we have not specifically examined the effects of more than one concussion on behaviour. Here we characterize the cognitive-motor integration performance of individuals as a function of concussion number.
We tested 199 asymptomatic individuals ages 9-53 years old (mean 18.71, 29.2% female) with a history of 1-10 concussions (mean 1.67). To assess CMI performance, participants were tested on 2 visuomotor tasks where reach and gaze were spatially dissociated using two linked touchscreens: 1) a standard condition requiring direct interaction with visual targets, and 2) a dissociated non-standard conditions requiring CMI (visual feedback reversal and motion/viewed targets in different planes). Based on the literature, we hypothesized that those with 2 or more concussions would experience significantly greater neuropathological effects on the brain networks required for rule-based visuomotor performance, relative to those with just 1. We predicted that this would translate to a significant worsening of behavioral performance in these individuals. An exploratory aspect of the study was how age, athletic experience, and sex would affect the concussion/performance relationship. We observed significant differences in both basic visuomotor ($p<0.05$) and CMI ($p<0.01$) performance, but paradoxically those in the 2+ concussion group performed *better*. We found significant correlations between both age (positive) and sport experience (negative), and visuomotor/CMI performance ($p<0.01$,both). We also observed these correlations for concussion group inclusion($p<0.01$). Thus, these data suggest that such factors have a greater impact on performance. Conclusions: Our predictions were not supported. Rather, because our sample was fairly young, these data emphasize the clinical importance of accounting for developmental age and sport experience when assessing the effects of multiple concussion on visuomotor skill.

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Poster

P530. Reaching Control: Motor Learning: Human Neurophysiology

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Program #/Poster #: P530.11

Topic: H.10. Human Learning and Cognition

Support: MH063901

Title: Changes in large scale brain organization during long term sequence learning

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Abstract: During learning, distributed brain systems change their functional interactions in order to satisfy the demands of mastering the task at hand. Over the course of learning, human fMRI studies have shown that the brain shifts to a more differentiated state in which brain systems work autonomously (Bassett et al., 2015; Finc et al., 2020). However, these studies have used relatively simple tasks, sampled over modest learning windows, which may incompletely capture long-term changes in large-scale network configuration. Here, we asked how large-scale brain organization would be impacted by intensive task-learning over a protracted training period. To answer this question, we scanned individuals repeatedly across three months, densely sampling the learning process while acquiring structured sequence knowledge in the context of a serial reaction time (SRT) task. In this task, novel images were embedded in probabilistic temporal sequences of four stimuli. Robust sequence learning was observed behaviorally via reduced response time for intact sequences across sessions. To quantify changes in the brain's large-scale organization, we evaluated functional connectivity based on the brain's "background" fluctuations i.e., correlated fluctuations that were slower than the transit trial evoked activation. We then assessed that background connectivity using modularity, a graph-theoretic quantity measuring the integrity of whole-brain differentiated communities. High modularity implies more segregation between communities. Using linear mixed modeling we found that modularity consistently increased in participants as a function of time, indicating that a more differentiated network organization developed across task acquisition. However, this effect emerged later in training, suggesting that modularity increased gradually with learning. We also examined modularity in a second learning phase, when a set of novel images were introduced into the SRT task to reset the learning process. Some subjects showed a dip in modularity at the start of the second phase, which then recovered and increased again across learning, while other subjects showed a continuous increase in modularity. This individual variability may reflect different levels of adaptation to the new cognitive demands. In conclusion, over the course of 3 months, we found evidence for changes in large-scale organization in which brain systems became more autonomous, which may enable automaticity in executing well-learned tasks. In the future we want to examine whether these changes in modularity are driven by changes in large-scale interactions between specific learning systems.

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Poster

P533. Interlimb and Bimanual Control

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Program #/Poster #: P533.01

Topic: E.04. Voluntary Movements

Support: 5R01HD059783-08

Title: Bimanual control policies that confer coupling between the limbs are dependent on the inertial symmetry of a given task.

Authors: *J. YUK[1], A. PRZYBYLA[2], R. L. SAINBURG[1];
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Abstract: Prior research on bimanual arm movements has shown that coordination depends on establishing a specific control policy or synergy in which potential errors arising from one hand can be efficiently compensated by the other. These control policies have been shown to recruit both feedforward and feedback mechanisms, which can yield remarkably symmetric temporal and spatial coupling between arms when required movements are mirror imaged and unperturbed. Coupling of bimanual movements through such control policies has been shown to be maximized when the task is shared between the arms (shared cursor condition), as compared to when the task is independent (independent cursor for each hand). Previous research has indicated that interlimb differences in coordination during reaching movements are associated with dominant arm advantage for coordinating intersegmental inertial dynamics. We now ask whether bilateral control policies associated with shared, as compared with independent task conditions reduce movement asymmetries through coupling of interlimb commands and feedback mediated actions. We designed a task with either symmetric or asymmetric intersegmental inertial requirements. We hypothesized that bimanual control policies established during the shared (single cursor) condition should reduce interlimb trajectory asymmetries, as compared with those during the non-shared condition (independent cursors). We found a strong spatial correlation and temporal synchrony of both arms when the two arms reached with symmetrical inertial conditions, but poor correlations and asynchronous actions when reaching with asymmetrical inertial conditions. This pattern was not affected by cursor condition (shared or independent), as we predicted. We conclude that bimanual control policies that confer interlimb coupling during bimanual tasks are dependent on the biomechanical symmetry of the task, and thus are not general in nature. Therefore, this type of bimanual coupling should be limited in the application to most common tasks, which do not involve mechanically symmetric actions.

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Poster

P533. Interlimb and Bimanual Control

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Topic: E.04. Voluntary Movements

Support: NIH Grant NS116886
NIH Grant NS061963

Title: Mouse motor cortex activity during the dexterous high-speed oromanual movements of food-handling

Authors: *J. M. BARRETT, A. FORREST, F. HAUSMANN, N. LONGO, M. E. MARTIN, W. MWANIKI, M. G. RAINERI TAPIES, M. TORRES, G. M. SHEPHERD;
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Abstract: The coordinated use of the hands and mouth to manipulate and consume food items is an ethologically relevant forelimb behavior exhibited by mice. Prominent features of this behavior include rapid cycling between a passive ‘holding’ mode and an active ‘oromanual’ mode, and ultra-fast ‘regrip’ maneuvers (Barrett et al, 2020). To explore the neural basis for this skillful behavior, we recorded from motor and somatosensory cortical regions during food handling.

High-speed (1000 fps) video was recorded while head-fixed adult mice of either sex handled and ate food items such as sunflower seeds and grain pellets. Kinematics were analyzed using DeepLabCut tracking. Recordings of single- and multi-unit spiking activity were made using 32-channel linear multielectrode arrays placed in forelimb motor cortex (fl-M1), forelimb somatosensory cortex (fl-S1), or tongue-jaw motor cortex (tj-M1; Mayrhofr et al.,2019).

In all three recorded regions, array-average firing rates were higher during the oromanual phase than during holding. However, the pattern of activity in each region was distinct. fl-M1 firing peaked around the transition into oromanual, then decayed slightly but remained elevated above baseline as handling continued. By contrast, fl-S1 and tj-M1 were both tonically active throughout the oromanual phase.

Array-average firing rates may mask neuron-to-neuron variation in patterns of food-handling-related activity. To investigate these, we clustered neural responses using non-negative matrix factorization (NNMF; Lee & Seung, 1999; Xu et al. 2020). In all three regions NNMF revealed two main clusters: one peaking in activity around the transition into the oromanual phase; and the other active later in the oromanual phase and peaking around regrips. Relatively more of the former type of response was seen in fl-M1, whereas fl-S1 and tj-M1 had proportionally more of the latter.

Our findings show that activity in sensorimotor cortex correlates with kinematic features of ongoing food-handling behavior, with patterns of activity that vary as a function of cortical area and between neurons within a given area. These results support the use of food-handling as a paradigm for studying neural mechanisms of complex, innate, dexterous behavior in the mouse.

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Poster

P533. Interlimb and Bimanual Control

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Program #/Poster #: P533.03

Topic: E.04. Voluntary Movements

Support: College of Education and Human Development Faculty Research Award from the University of Texas at San Antonio

Title: Interactions between discrete and rhythmic movements in bimanual upper-limb control

Authors: R. ANGEL, *S.-W. PARK;
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Abstract: Many motor tasks in everyday life, such as driving and cooking, involve a combination of discrete and rhythmic movements. Although an increasing number of studies have identified discrete and rhythmic movements as dynamic primitives in complex motor control, specifying conditions in which rhythmic movements facilitate discrete movements and vice versa remains unknown. The present study measures the change of kinematics when ongoing rhythmic movement in one arm is perturbed by rhythmic or discrete initiation of the other arm.Thirteen healthy young adults participated in the study after giving a written informed consent approved by the Institutional Review Board. The participants performed bimanual forearm rotation on the horizontal plane. In one condition (DISCRETE), their non-dominant arm initiated a point-to-point reaching while their dominant arm moved rhythmically at a specific tempo (0.75 Hz). In the other condition (RHYTHMIC), their non-dominant arm initiated a rhythmic movement while their dominant arm performed the same rhythmic movement as in the first condition. For both conditions, timing of the imperative visual cues for initiation was randomized. The elbow angle profile from the two forearm rotations was recorded by absolute encoders so that the veridical elbow angle was displayed on a monitor in real time. The task consists of two phases: Familiarization and test. In the familiarization phase, the participants practiced moving their forearm at a specific speed (180 deg/s) for the discrete movement and at a specific tempo (0.75 Hz) for the rhythmic movement unimanually and then bimanually. In the test phase, approximately 100 trials for the DISCRETE condition (50 inward and 50 outward movements) and 100 trials for the RHYTHMIC condition were performed for each participant. We analyzed the magnitude and direction of the perturbation in the ongoing rhythmic movement in the dominant arm as calculating the root-mean-squared error between the desired and actual profile of instantaneous phase in the dominant arm at the initiation of the non-dominant arm.

Results showed that, when a rhythmic movement is initiated, the amount of perturbation in the ongoing rhythmic arm depended on the phase relationship between the two arms. When a discrete movement is initiated, the amount of perturbation in the ongoing rhythmic arm depended on the phase of the ongoing arm at the initiation of the discrete movement. Interestingly, this was independent from the direction of the initiation of the discrete arm. The results suggest that the underlying neural mechanism in discrete movement is different from that in rhythmic movement.

Disclosures: R. Angel: None. **S. Park:** None.

Poster

P533. Interlimb and Bimanual Control

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Program #/Poster #: P533.04

Topic: E.04. Voluntary Movements

Support: SEC Faculty Travel Grant

Title: The effects of bimanual coordination constraints on postural control

Authors: *J. M. HONDZINSKI[1], M. DAVIS[2], Y. WANG[2], R. CASTRO, Jr.[2], R. HUA[2,3], D. M. KENNEDY[2];
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Abstract: Many activities of daily living require individuals to coordinate actions with their upper limbs while standing. A coalition of bimanual coordination constraints have been identified that influence the impact of one limb on the contralateral limb, including cortical and subcortical neural crosstalk. However, it is unclear if these bimanual constraints also impact balance. Therefore, the current experiment was designed to determine the effects of bimanual coordination constraints on postural control. Eleven right limb dominant participants (mean age = 20.9, 6 Female, 5 male) were required to rhythmically produce patterns of isometric force by abducting their index fingers in a 1:1, 1:2, and 2:1 coordination pattern while standing on a force plate. Lissajous displays and a goal template were used to guide performance. The template illustrated the specific pattern of force requirements needed to produce the coordination patterns. Participants performed 20, 35 second trials in 2 blocks for each coordination pattern at 10% maximum voluntary contraction (MVC). Results indicated the participants could quickly and effectively perform the bimanual coordination patterns within the allotted trials. As expected, bimanual performance was more accurate and stable for 1:1 compared to 1:2 and 2:1 patterns. When comparing the 1:2 and 2:1 tasks, the results indicated that the dominant limb influenced the non-dominant limb regardless of which limb was performing the faster frequency; however, the non-dominant limb only influenced the dominant limb when assigned the faster frequency.

This influence is also reflected in the harmonicity values with decreased harmonicity for the left limb in the 1:2 task and for both the left and right limb in the 2:1 task. A decrease in harmonicity indicates an increase in adjustments, hesitations, and/or perturbations. Similarly, the postural sway data indicated that participants were more stable during the 1:1 task than the 1:2 and 2:1 tasks. When performing the 1:2 and 2:1 bimanual coordination tasks greater medial-lateral sway displacement was observed than for the 1:1 task. However, no differences were observed in the anterior-postural direction during the bimanual tasks. These results are consistent with previous investigations indicating the neural crosstalk can influence bimanual coordination by stabilizing 1:1 coordination and interfering with other frequency patterns. Interestingly, it appears that the motor, perceptual, and/or cognitive constraints associated with bimanual coordination can also influence postural control.

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Poster

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Topic: E.04. Voluntary Movements

Support: NIH Grant R01HD059783

Title: Deficits in performance on a mechanically coupled bilateral task in chronic stroke survivors with mild unilateral paresis

Authors: *S. A. L. JAYASINGHE[1], C. MAENZA[1,2], D. GOOD[1], R. L. SAINBURG[2,1]; [1]Neurol., Pennsylvania State Univ. Col. of Med., Hershey, PA; [2]Kinesiology, Pennsylvania State Univ., State College, PA

Abstract: Most upper extremity mediated activities of daily living require coordinated interaction between the arms, such as when buttoning a shirt or cutting a slice of bread. Previous work using bilateral reaching tasks performed either synchronously or asynchronously have provided evidence of bilateral coordination deficits in stroke survivors. However, these approaches have typically used movements that do not reflect the distribution of functions that occur in everyday tasks, nor have they addressed the natural type of mechanical coupling that occurs between the arms in such tasks. We now present a virtual reality-based task that incorporates mechanical coupling, requiring that one hand stabilize against a spring load that varies with the displacement of the other arm. We examined differences in performance between the ipsilesional and contralesional hands in these two aspects of control in 15 unilateral chronic stroke survivors with mild contralesional impairment. We also examined differences between

hands in 7 age-matched typical adult controls. All participants were right-handed. The hands were connected by a spring, and participants had to maintain the position of one hand while using the other hand to perform a rapid out-and-back movement. We present two primary performance measures to reflect the positional stability of the stabilizing hand (acceleration and maximum distance moved) and accuracy and coordination of the reaching hand (initial direction error and linearity). For reaching performance, we found that performance was more erroneous in our stroke group than in the control group. We found a main effect of group for linearity ($F(1,20)=6.57$, $p=.019$), and post hoc analysis revealed significant differences between hands in stroke survivors ($p=.047$). We found a main effect of group for initial direction error as well ($F(1,20)=4.36$, $p=.0498$). For stabilizing performance, we found a significant interaction between hand and group for acceleration ($F(1,20)=6.75$, $p=.017$), such that the dominant right hand of controls accelerated more than the nondominant left hand. While the spring forces imposed by motion of the reaching hand produced interaction effects with regards to acceleration, these effects were not significant for distance. Our findings suggest that when remediating bilateral movements in stroke, using the ipsilesional arm for manipulation while using the contralesional arm for stabilizing might be an effective strategy. However, more research is necessary to determine whether this strategy can be effective when it is opposite to the pattern used premorbidly, due to handedness.

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Poster

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Topic: E.04. Voluntary Movements

Support: NIH Grant R01HD082216

Title: Repeated locomotor adaptation and de-adaptation facilitate retention of motor learning during walking in individuals post-stroke

Authors: *S. PARK[1,2], S. YAN[1,2], W. DEE[1], R. REED[1], E. ROTH[1,2], W. RYMER[1,2], W. MING[1,2,3];
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Abstract: Gait restoration has been one of the important parts of stroke rehabilitation. Inducing continuous adaptation to new walking patterns has been widely used for rehabilitation of gait after stroke. However, walking is already a complex task and the adaptation to novel walking

patterns with no/minimal rest during a single session may be too physically demanding to efficiently promote the locomotor learning. Here, we test whether the spaced-out adaptation (i.e., repeated adaptation and de-adaptation) to novel walking patterns elicited by external perturbation induces better retention of the adapted locomotion in stroke survivors, compared with the effect of the continuous adaptation. Fifteen individuals post-stroke (58.5±6.0 years, 6 women) participated in two experimental sessions consisting of 1) treadmill walking inducing spaced or continuous adaptation to externally perturbed walking patterns and 2) overground walking. During the treadmill walking, we applied the laterally pulling force to the pelvis toward the non-paretic side during the stance phase of the paretic leg to disturb mediolateral weight shifts toward the paretic side. Both spaced and continuous condition elicited improved weight shift toward the paretic side and enhanced muscle activation of hip abductor and adductors immediately after the removal of the pelvis perturbation ($P<0.05$). Only the spaced condition retained these improved weight shift and enhanced muscle activation later after the perturbation release and even exhibited increased overground walking speed after the treadmill walking ($P<0.04$). We provide new evidence that spaced adaptation to novel locomotor patterns reinforces retention of the adapted locomotion and its generalization to the other context, compared with the effect of the continuous adaptation.

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Poster

P533. Interlimb and Bimanual Control

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Program #/Poster #: P533.07

Topic: E.04. Voluntary Movements

Support: TL1TR001431
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1U10NS086513-01

Title: Feasibility of a virtual reality based upper extremity rehabilitation platform

Authors: *A. BRUNFELDT[1], P. LUM[2], B. BREGMAN[1], A. W. DROMERICK[3]; [1]Georgetown Univ., Washington, DC; [2]The Catholic Univ. of America, Washington, DC; [3]Natl. Rehab Hosp, Washington, DC

Abstract: Translational researchers are intensely focused on developing safe and effective therapies for people with motor impairments. Recent advances in upper extremity stroke rehabilitation have failed to show sustained improvements. This is likely due to therapies either costing substantial time and money, or they lack resemblance to activities of daily living. To

improve health outcomes for patients, we need ecologically valid movement tasks capable of increasing patients' use of their paretic limb. Here we developed a virtual reality (VR) reaching task using commercially available equipment to systematically alter bilateral coordination to favor the use of one limb over the other.
We conducted a feasibility study in healthy, right-handed volunteers where they performed a bimanual reaching task in a 3-dimensional VR environment. They controlled a shared cursor, located at the midpoint between the hands, and reached to targets located at 80% of full arm extension. Participants completed a total 480 reaches divided into 5, 96 trial blocks. In the first, third, and fifth blocks, participants reached normally. In the second and fourth blocks they reached with a wrist weight applied to one limb. We used surface EMG to approximate muscle activity in the anterior deltoid and biceps brachii, bilaterally. Relative contribution (RC) was calculated as the right hand displacement divided by the sum of right and left hand displacements. The root mean square (RMS) of surface EMG was used to calculate muscle contribution (MC), computed as RMS in the right muscle divided by the sum of RMS in both muscles (per pair).
We found that during the first baseline block, participants reached with an equal contribution from both arms (RC = 50%). RC increased by 0.91% and MC decreased by 5.6% relative to baseline when the weight was applied to the left arm; RC decreased by 0.94% and MC increased by 6.7% when the weight was applied to the right arm. These data suggest participants alter coordination in an attempt to minimize muscle output asymmetry between limbs. Our task can be paired with assistive devices that normalize muscle output asymmetries between the upper extremities in hemiplegic stroke patients. This may form the basis for low cost, at-home technologies for stroke rehabilitation.

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Poster

P533. Interlimb and Bimanual Control

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Program #/Poster #: P533.08

Topic: E.04. Voluntary Movements

Support: 5R01HD059783-08

Title: The flexibility vs persistence of visual-motor control policies (synergies) for coordinating bilateral arm movements

Authors: *N. M. KITCHEN[1,2], R. L. SAINBURG[2,1], A. PRZYBYLA[3];
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Abstract: Prior research on bimanual arm movements has shown that coordination depends on establishing a specific control policy or synergy in which potential errors arising from one hand can be efficiently compensated by the other. These control policies have been shown to recruit both feedforward and feedback mechanisms, which results in remarkably symmetric temporal and spatial coupling between arms when required movements are mirror imaged and unperturbed. In contrast, when the required movements of the two arms differ, decoupling can be remarkably difficult and lead to bilateral interference. Thus, previous evidence suggests that bimanual control policies can either facilitate or degrade coordination, depending on task demands. Here, we address the flexibility vs persistence of visual-motor control policies for coordinating bilateral movements. Participants performed a series of bimanual reaching movements to transport a single, shared cursor from a start position to a target along the mid-sagittal axis. On different blocks of trials, the gain contribution of either hand to the cursors horizontal position (i.e. perpendicular to start-target axis) was covertly manipulated to be either equal (2:2 ratio) or asymmetric (3.8:0.2 ratio) between hands. We predicted that an inflexible bimanual control policy would be unaffected by asymmetric gains such that covariance would be maintained between the hands, while a compliant policy should adjust to the asymmetric gain conditions, reducing covariance. We found a significant change in the regression slope for the asymmetric gain conditions, consistent with a compliant bimanual control policy. However, this effect was significant only at movement end and was incomplete, maintaining some covariance under conditions where it was of no benefit to task accuracy. Furthermore, these changes in control policy appeared implicit because modifications of gain were covert, and participants did not report awareness of the gain manipulation following the experiment. Thus, visual-motor control policies for coordinating bilateral movements respond flexibly to task demands but this adaptability is limited by persistence of bimanual coupling.

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Poster

P533. Interlimb and Bimanual Control

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Program #/Poster #: P533.09

Topic: E.04. Voluntary Movements

Title: Human-human connected dyads learning a visuomotor rotation in a movement tracking task

Authors: *A. GENDY[1], J. L. PATTON[2], M. DEMASI[1];
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Abstract: Dyads are 2 humans coupled to collaborate to perform a task together. Several studies have shown that mechanical haptic interactions lead to the dyad outperforming the solo task. These achievements are hypothesized to be the result of haptic communication, triggering the creation of internal forward models. The dyad acquires and exploits additional information about itself, improving performance. Here we show a novel Pantograph device used in a pilot study to further study this learning process. Like other studies, seated subjects practiced a pursuit task for 40 minutes holding the pantograph handles with visual feedback from a flatscreen superimposed above their arms. We hypothesized that Novice-Novice interaction enhances learning speed more than Expert-Novice, since it requires mutual negotiation. We found this was unsupported (learning time constants 5.53 ± 2.79 for Novice-Novice and 8.45 ± 3.78 for Expert-Novice, p = .075). This study demonstrates how a simple device built in-house for haptic interaction between humans can further reveal the characteristics of collaborative learning processes.

Disclosures: A. Gendy: None. **J.L. Patton:** None. **M. Demasi:** None.

Poster

P538. Cortical Planning and Execution: Premotor and Motor Cortex: Neurophysiology: Animal III

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P538.01

Topic: E.04. Voluntary Movements

Support: UH3NS107714
U01NS108922
R01 NS045853
R01 NS111982
NDSEG Fellowship

Title: Longevity of the Utah intracortical multi-electrode array: a cross-species analysis

Authors: *C. SPONHEIM[1], V. PAPADOURAKIS[1], J. L. COLLINGER[2], J. DOWNEY[1], J. M. WEISS[3], L. PENTOUSI[4], K. ELLIOTT[5], N. HATSOPOULOS[1];
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Abstract: Microelectrode arrays are standard tools for conducting chronic electrophysiological experiments, allowing researchers to simultaneously record from large numbers of neurons. Specifically, Utah electrode arrays (UEAs) have been utilized by scientists in many species, including rodents, rhesus macaques, marmosets, and human participants. The field of clinical human brain-computer interfaces currently hinges on using the UEA as several research groups

have FDA clearance through the investigational device exemption pathway. Despite its widespread usage in systems neuroscience, few studies have comprehensively evaluated the reliability and signal quality of the Utah array over long periods of time in a large dataset. We collected and analyzed over 6000 recorded datasets from various cortical areas spanning almost a decade of experiments. The scale of this dataset allowed us to evaluate the average life of these arrays, based primarily on the signal-to-noise ratio of each electrode over time. Using implants in multiple motor-related cortices, we found that the average lifespan of available recordings from UEAs was over one and a half years, although we provide several examples of these UEAs lasting over 1000 days and one up to 9 years; human implants were also shown to last longer than non-human primate implants. We also found that electrode length did not affect longevity and quality, but iridium oxide metallization on the electrode tip exhibited superior longevity and quality as compared to platinum metallization.

Disclosures: C. Sponheim: None. **V. Papadourakis:** None. **J.L. Collinger:** None. **J. Downey:** None. **J.M. Weiss:** None. **L. Pentousi:** None. **K. Elliott:** None. **N. Hatsopoulos:** F. Consulting Fees (e.g., advisory boards); Blackrock Microsystems.

Poster

P538. Cortical Planning and Execution: Premotor and Motor Cortex: Neurophysiology: Animal III

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P538.02

Topic: E.04. Voluntary Movements

Title: Temporal network dynamics of cortical neurons reveal single-trial task information in the progression of pairwise correlations

Authors: *M. SUNDIANG, N. G. HATSOPOULOS, J. N. MACLEAN;
Univ. of Chicago, Chicago, IL

Abstract: Neural dynamics in primary motor cortical (MI) populations underlie skillful, voluntary movements. Linking these population dynamics to individual neurons is key to understanding how MI drives complex behaviors. To do so we summarize pairwise correlations between neurons by representing population dynamics as a functional network (FN). Typically FNs are generated on a trial by trial basis. However since movement is inherently a dynamic process, here we capture the temporal progression of these pairwise correlations within small epochs of time throughout the trial in order to accurately relate single-trial population dynamics to behavior. We constructed FNs by measuring the estimated mutual information between spike trains in macaque MI within a sliding window across single-trials in a center-out reaching task. To examine the temporal progression of the networks over the course of a single trial, temporal FNs were projected into a low-dimensional space using UMAP. This low-dimensional

embedding revealed that the dynamically evolving trajectories of the single-trial temporal FNs in this space are separated and carry information about the instructed reach. Additionally, we measured the network similarity (graph alignment score, GAS) between all pairs of FNs and show that the similarity of these temporal FNs depend on the similarity of the reach, and become maximally dissimilar when we compare opposite reach directions. To determine how discriminable the instructed reach is using single-trial FNs, we measured the fraction of k-nearest-neighbors in the high-dimensional space belonging to the same reach direction using a precision-at-k (p@k) metric. We observe a significant increase p@k of FNs constructed from the data compared to FNs constructed from rate-matched Poisson neurons indicating additional information beyond firing rates alone, again, highlighting the importance of pairwise correlations in decoding behavior. These results demonstrate that FNs carry behaviorally relevant information and that they are dynamic over time which argues for a temporal network perspective to link neural dynamics and network structure to complex behavior.

Disclosures: M. Sundiang: None. **N.G. Hatsopoulos:** None. **J.N. MacLean:** None.

Poster

P538. Cortical Planning and Execution: Premotor and Motor Cortex: Neurophysiology: Animal III

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Program #/Poster #: P538.03

Topic: E.04. Voluntary Movements

Support: NINDS Grant R01-NS-104898

Title: Characterizing neuromechanical strategies used by marmosets during foraging and prey capture

Authors: *J. WALKER[1], J. N. MACLEAN[3], N. G. HATSOPOULOS[2];
[1]Organismal Biol. and Anat., [2]Univ. of Chicago, Chicago, IL; [3]Neurobio., The Univ. of Chicago, Chicago, IL

Abstract: Understanding the cortical contribution to motor control will benefit from investigations of motor cortical neural dynamics during complex naturalistic behaviors (Saxena & Cunningham 2019; Gao & Ganguly 2015; Gao et al. 2017; Musall et al. 2018). Foraging and prey capture are ideal behaviors to study as they are ethologically relevant and provide natural extensions of standard experimental tasks used to study motor control and learning (Borghuis & Leonardo 2015; Hein et al 2020; Yoo et al. 2020). Here we apply a voluntary behavioral training approach to allow common marmosets (Callithrix jacchus) daily sessions of foraging (mealworms) and prey capture (moths and crickets) over the course of multiple months. We use markerless motion capture to reconstruct the kinematics of the marmoset's visuo-manual

behavior and wireless multi-electrode array recordings to simultaneously sample sensorimotor neocortical populations from premotor cortex, primary motor cortex and primary sensory cortical areas. We present preliminary estimates of kinematic distributions and sensorimotor delays characterizing marmoset foraging and prey capture toward a description of neuromechanical strategies employed during these naturalistic visuo-manual behaviors.

Disclosures: J. Walker: None. **J.N. MacLean:** None. **N.G. Hatsopoulos:** Other; discounts on products as a consultant; Blackrock Microsystems.

Poster

P538. Cortical Planning and Execution: Premotor and Motor Cortex: Neurophysiology: Animal III

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Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P538.04

Topic: E.04. Voluntary Movements

Support: NIH R01 Grant: R01MH123770

Title: Dissociating intrinsic and input-driven neural dynamics underlying behavior

Authors: *P. VAHIDI, O. G. SANI, M. M. SHANECHI;
Electrical and Computer Engin., USC, Los Angeles, CA

Abstract: To understand the neural population dynamics underlying behaviors, it is crucial to study the source from which these dynamics emanate. Neural dynamics observed in a brain region could either be largely due to the intrinsic dynamics of the recurrent networks within that region, or reflect the dynamics of the inputs to that region. These inputs could be excitations from other brain regions or even from sensory stimuli. Since inputs themselves could be temporally structured, a major challenge is faced when building models of neural dynamics: these models may misinterpret input dynamics as intrinsic dynamics emanating from the recurrent networks within the brain region. As a result, researchers may be limited in what conclusions they can make from these models and may need to design careful experiments — for example by only using external sensory inputs that are not temporally structured. Further, whether input-driven or intrinsic, neural population dynamics relate to a diversity of behaviors and functions that happen simultaneously; thus, it is critical to dissociate those dynamics that are relevant to a particular measured behavior of interest (e.g. limb movement kinematics) so that they are not masked or confounded by other dynamics. Recently, we developed a new method called preferential subspace identification (PSID) that dissociated behaviorally relevant neural dynamics but without explicit modeling of inputs (Sani et al, Nat Neurosci, 2021). Here, we develop a new modeling approach termed input-PSID (IPSID), that addresses both challenges simultaneously. Our new approach enables dissociation of intrinsic neural dynamics from the

dynamics of measured inputs (e.g. sensory task instructions), and hence avoids learning models that are confounded by sensory input dynamics. IPSID also models the effect of input on neural activity and prioritizes the learning of behaviorally relevant neural dynamics, whether input-driven or not, such that they are not masked by other neural dynamics. We first demonstrate in numerical simulations that IPSID correctly dissociates intrinsic dynamics from input dynamics. We next apply IPSID to neural activity recorded from monkeys during behavioral tasks. We find that IPSID learns distinct neural dynamics and intrinsic modes of activity that are not confounded by sensory input dynamics. Moreover, models learned by IPSID more accurately explain behaviorally relevant neural dynamics compared with prior modeling approaches. IPSID uniquely enables the dissociation of input dynamics from intrinsic behaviorally relevant neural dynamics to study the neural basis of behavior.

Disclosures: P. Vahidi: None. **O.G. Sani:** None. **M.M. Shanechi:** None.

Poster

P538. Cortical Planning and Execution: Premotor and Motor Cortex: Neurophysiology: Animal III

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Topic: E.04. Voluntary Movements

Support: NIH Director's New Innovator Award DP2-MH126378
ARO MURI W911NF-16-1-0368

Title: Learning behaviorally relevant dynamics in population spiking activity with Poisson preferential subspace identification (Poisson-PSID)

Authors: *L. L. OGANESIAN, O. G. SANI, M. M. SHANECHI;
Electrical Engin., USC, Los Angeles, CA

Abstract: Population spiking activity consists of a large number of binary time-series happening at fast millisecond time-scales and indicating the presence or absence of action potential events. This activity can simultaneously represent a mixture of numerous cognitive processes and behaviors, leading to two challenges when modeling the neural encoding of behavior. First, encoding models need to describe the temporal structure of both behavioral and spiking activity, the latter being a collection of binary time-series. Second, encoding models need to dissociate the behaviorally relevant information in spiking activity, defined as all the information related to a particular measured behavior of interest, from other information. Algorithms that learn state-space models with linear-nonlinear Poisson observations for population spiking activity do not address the second challenge because they are unsupervised with respect to behavior. We recently developed the preferential subspace identification (PSID) algorithm that solves both

challenges for continuous neural observations, such as field potentials or binned spike counts (Sani et al, Nat Neurosci, 2021). However, dissociating behaviorally relevant dynamics while taking into account the discrete binary nature and fast timescale of spiking activity introduces the new challenge of finding shared subspaces between hybrid continuous and discrete signals. Here we introduce a new learning algorithm termed Poisson-PSID that addresses this new challenge by jointly modeling both continuous behavioral and discrete population spiking activity in terms of low-dimensional latent states, while dissociating and prioritizing behaviorally relevant spiking dynamics. We show that Poisson-PSID can successfully learn low-dimensional models of spiking dynamics that preserve behaviorally relevant information and decode behavior more accurately. This new Poisson-PSID algorithm can be used to uncover low-dimensional behaviorally relevant manifolds in population spiking activity, helping reveal the neural basis of diverse behaviors across brain regions and enabling better brain-machine interfaces.

Disclosures: L.L. Oganesian: None. **O.G. Sani:** None. **M.M. Shanechi:** None.

Poster

P538. Cortical Planning and Execution: Premotor and Motor Cortex: Neurophysiology: Animal III

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Program #/Poster #: P538.06

Topic: E.04. Voluntary Movements

Support: ONR Young Investigator Program: N00014-19-1-2128
NIH R01: R01MH123770

Title: Nonlinear modeling of behaviorally relevant neural dynamics using recurrent neural networks

Authors: *O. G. SANI[1], B. PESARAN[4], M. M. SHANECHI[1,2,3];
[1]Electrical and Computer Engin., [2]Neurosci. Grad. Program, [3]Biomed. Engin., USC, Los Angeles, CA; [4]Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Neural activity exhibits diverse dynamics that simultaneously relate to multiple brain functions and behaviors. Thus, a significant challenge when studying the neural basis of a specific measured behavior is that the dynamics related to that behavior may be masked or confounded by all the other neural dynamics that coexist. Recently, we developed a method termed preferential subspace identification (PSID) that addresses this challenge (Sani et al, Nat Neurosci, 2021) by modeling behavior and neural activity jointly while prioritizing the learning of their shared dynamics. While PSID addresses the challenge of learning behaviorally relevant neural dynamics within a linear modeling framework, studies suggest that neural dynamics, including potentially behaviorally relevant dynamics, could be nonlinear. Here, we develop a

new method using recurrent neural networks (RNN) that learns nonlinear dynamical models with low-dimensional latent states that can predict behavior and neural activity, while prioritizing preservation of shared dynamics and states between behavior and neural activity. As a result, this method also enables nonlinear dimension reduction for neural activity in a way that preserves behaviorally relevant neural dynamics. We validate the method in numerical simulations and then use it to explore nonlinearities in behaviorally relevant neural dynamics in multiple experiments that record neural activity in monkeys performing different behavioral tasks. We find that this nonlinear method extracts low-dimensional latent states from neural activity that more accurately predict behavior compared to linear PSID and nonlinear dynamic modeling approaches that do not prioritize behaviorally relevant information. Further, using the new method, we find that across multiple experiments, similar model parameters are consistently the most important in capturing the nonlinear behaviorally relevant neural dynamics. Finally, we demonstrate that models learned by the new method achieve better neural decoding of behavior compared with other methods while still being fully causal and real-time. Overall, the novel method introduced here enables flexible nonlinear dynamical modeling and dimension reduction of neural population activity while preserving behaviorally relevant information and enabling causal real-time behavior decoding.

Disclosures: O.G. Sani: None. **B. Pesaran:** None. **M.M. Shanechi:** None.

Poster

P538. Cortical Planning and Execution: Premotor and Motor Cortex: Neurophysiology: Animal III

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Topic: E.04. Voluntary Movements

Support: DoD Bilateral Academic Research Initiative (DoD BARI), grant number W911NF1810434

Title: Efficient learning of low dimensional latent dynamics in multiscale spiking and field potential population activity

Authors: *P. AHMADIPOUR, O. G. SANI, Y. YANG, M. M. SHANECHI;
Electrical and Computer Engin., USC, LOS ANGELES, CA

Abstract: Learning low-dimensional latent state representations in spike and field potential activity together can reveal the relationships between population dynamics across different spatiotemporal scales and how they explain behavior. But because spikes are discrete-valued action potential events with millisecond time-scales while field potentials are continuous-valued with slower time-scales, their simultaneous multiscale modeling had remained elusive. We

recently resolved this challenge by developing a multiscale expectation maximization (multiscale EM) algorithm that maximizes the joint likelihood of these signals iteratively, and discovers a multiscale low-dimensional dynamical mode that is shared in spike-field activity and best explains naturalistic movements (Abbaspourazad et al, Nature Communications, 2021). However, in some applications -- for example when tracking adaptation and plasticity in basic science studies or during real-time learning in brain-machine interfaces --, a desirable feature for a learning algorithm is computational efficiency. Consequently, it is critical to develop multiscale learning methods that are more computationally efficient than the multiscale EM, which is iterative. Here, we develop a multiscale subspace identification (multiscale SID) algorithm that enables computationally efficient multiscale learning and dimensionality reduction for simultaneous spike-field activity. We demonstrate that the new multiscale SID accurately learns dynamical models of spike-field signals, extracts multiscale low-dimensional dynamics/modes in these signals, and decodes behavior. Further, it correctly aggregates information from spikes and field potentials, leading to better identification of multiscale dynamics/modes and decoding of behavior compared to using a single scale. Most critically, multiscale SID achieves the above precise learning, dimensionality reduction, and decoding with much lower computational cost compared with multiscale EM while having better or similar accuracy. Overall, multiscale SID is beneficial both for studying multiscale neural dynamics and for brain-machine interfaces, especially when efficient and real-time tracking is important such as in learning and adaptation experiments or in brain-machine interfaces.

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Poster

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Title: Cortical motor maps depending on learning- and contexts

Authors: *K. TAMURA, H. THIALLIER, S. CROCHET, C. C. H. PETERSEN;
École Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland

Abstract: Cortex contributes to motor control, but cortical motor maps remain relatively poorly-defined. Here, we have begun to investigate whether cortical motor maps can change depending on learning and context. To quantitatively map causal behavioral impact, we optogenetically stimulate the dorsal cortex of head-fixed mice by focal laser pulses, and analyze the evoked orofacial movements by high-speed video filming. In naïve Thy1-ChR2 mice, stimulation in different coordinates evokes movements in different facial parts such as whiskers, jaw, nose and eyelids. Movements of the jaw are robustly evoked, but tongue protrusions are less apparent. Next, the mice are water-restricted and encounter a task in which they are sometimes unpredictably rewarded by voluntarily licking a spout. We perform optogenetic stimulation during inter-trial-intervals, and we do not reward the licks following the optogenetic stimulation. Unexpectedly, optogenetic stimulation now appears to evoke rhythmic protrusion of the tongue. The corresponding tongue motor map appears to show a focal spot in the area where jaw movements are also evoked, suggesting overlap of tongue and jaw primary motor cortex (tjM1). We next perform the motor mapping in the same mice after they have learned to lick the reward spout in response to a brief deflection of a single whisker. As a result, the tongue motor map appears to extend more posteriorly from tjM1 to somatosensory cortices. These results suggest that cortical motor maps are plastic and adapt depending on task demand and context. To further analyze the dynamics of cortical motor maps and the underlying circuit mechanisms, we are now studying layer-specific ChR2 mice during context-discrimination tasks combined with wide-field calcium imaging.

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Poster

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Title: Isolation of learned skills in motor cortex in a context-switching task

Authors: E. M. TRAUTMANN[1], N. J. MARSHALL[2], H. CHEN[4], E. ALIYARI[5], E. AMEMATSRO[5], D. M. WOLPERT[3], M. N. SHADLEN[6], M. M. CHURCHLAND[2]; [1]Zuckerman Inst., Columbia Neurosciences, New York, NY; [2]Neurosci., [3]Columbia Univ., New York, NY; [4]Columbia, New York, NY; [5]Columbia, NEW YORK, NY; [6]Neurosci., Howard Hughes Med. Inst. - Columbia Univ., New York, NY

Abstract: The brain can acquire a vast repertoire of motor skills, each requiring its own sensorimotor transformations. The motor cortex (M1) performs computations to control muscle activity, including error correcting responses. Are these computations skill-specific, or do they lie downstream of the library of motor skills? Put differently, if two skills require identical patterns of muscle activity, does M1 use different strategies or a unified strategy? In support of a unified strategy, pro- and anti-reaches are generated by similar M1-activity patterns. Yet this case is ambiguous — the sensory-motor transformations could be accomplished well before the reach. We thus sought to compare two skills that required different sensorimotor mappings throughout the movement, yet produced the same muscle activity. We trained a monkey to follow a scrolling path with a cursor using isometric forces. We employed two 'skill contexts', in which the cursor either moved up or down with increasing force. The same forces were generated in both contexts, driven by paths with inverted shapes. Using primate Neuropixels, we recorded large populations of neurons from M1 and dorsal premotor cortex. Most neurons' activity was strongly context dependent. A context-independent (force-predictive) neural dimension captured a modest fraction of the population variance. In most dimensions, activity was unrelated to force and was strongly context-dependent. We hypothesized that context-dependent activity enables transformation of the same visual error into different motor corrections. We introduced visual cursor perturbations, and found two dimensions that captured perturbation specific responses. In the first, transient activity reflected the sign of the visual perturbation. In the second, activity was more sustained and reflected the sign of the motor response. We speculate that this can occur because cortical dynamics allow activity in the 'visual' dimension to impact activity in the 'motor' dimension. Such dynamics have to be context-dependent, because the sign of the sensory-motor transformation reverses between contexts. This is consistent with the fact that cortical activity was strongly context dependent when the perturbation was delivered, allowing different local dynamics to implement the visual-to-motor transformation. These results suggest that neural activity driving feedback control in motor cortex is skill-specific. Initially this strategy seems implausible: it requires unique patterns of activity for every skill that maintain the necessary low trajectory tangling. Yet this is sensible given recent findings that M1 activity is surprisingly high dimensional.

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Poster

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Topic: E.04. Voluntary Movements

Support: Brain Research Foundation BRFSG-2019-08 (PI Yttri). Title: Revealing How Value is Translated into Action with Simultaneous Single Neuron and Multi-Area Resolution
Whitehall Foundation Research Grant (PI Yttri). Title: Cortico-Striatal Interactions Underlying Motor Decisions

Title: Motor cortex but not parietal lesions severely impair reaching, spontaneous motor decisions and striatal dynamics

Authors: *M. A. NICHOLAS[1,2], E. A. YTTRI[1,3];
[1]Biol. Sci., [2]Ctr. for the Neural Basis of Cognition, [3]Neurosci. Inst., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: What does the motor cortex contribute to behavioral decisions, performance and subcortical dynamics? Motor cortex and the basal ganglia may work together to plan movements, potentially through the generation of motor plans and control of kinematic parameters, respectively. This hypothesis has clear and testable predictions about the contribution of motor cortex to striatal dynamics and animal behavior. We sought to study movement kinematics during spontaneous and goal-directed behavior before and in the days immediately following bilateral lesions of the caudal forelimb area (CFA). This behavioral monitoring was paired with recordings from MSN and FSI neural populations in striatum. Mice were trained on a basic or reaction time-based reaching task. In both tasks, movement kinematics were severely impaired following ablation and began to recover towards baseline performance after 6-8 days.
Reaction time drastically increased and then return to pre-ablation levels. Concurrent recordings in striatum revealed a selective loss of tuning to movement onset and kinematics in both MSN and FSI populations, with severely altered response arising only after behavioral recovery.
Additionally, we found that while locomotion was largely intact after lesions, however when faced with a decision (e.g. to turn left or right) the animal paused in place for several tens of seconds in a fashion similar to freezing of gait seen in Parkinson's disease.
Finally, we contrasted these result to lesion of posterior parietal cortex, which did not induce widespread changes in striatal activity or behavior and served as a control against generalized brain dysfunction following lesion.
Our results help to understand how neural populations orchestrate both goal-directed and spontaneous, naturalistic behavior. As striatal tuning to movement kinematics is lost even after reaching behavior recovers, these results argue strongly that cortex selects actions, changing between motor states, and drives the movement-related dynamics in striatal neurons.

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

Poster

P538. Cortical Planning and Execution: Premotor and Motor Cortex: Neurophysiology: Animal III

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Program #/Poster #: P538.11

Topic: E.09. Motor Neurons and Muscle

Support: 5R01NS111982

Title: Movement-specific spatial recruitment patterns in the primary motor cortex

Authors: *W. LIANG[1], K. BALASUBRAMANIAN[1], V. PAPADOURAKIS[2], N. G. HATSOPOULOS[1];
[2]Organismal Biol. and Anat., [1]Univ. of Chicago, Chicago, IL

Abstract: The primary motor cortex (M1) is known to have a coarse spatial organization where representations of different body parts form clusters on the cortical sheet. However, it is largely unknown if there exists any spatiotemporal organization that is behaviorally relevant. Our previous work found that amplitude attenuation of the local field potential (LFP) in the beta band (13-35Hz) propagates along a stereotyped rostro-caudal axis and signals the initiation of movement but does not provide movement-specific information [1]. To characterize potential M1 spatiotemporal patterns that might be important during movement execution, this study investigated the high gamma band (200Hz - 400Hz) of the LFP given its similar directional tuning properties to multi-unit activity as well as its established utility in movement decoding for brain-machine interfaces [2]. Neural recordings were collected from two male rhesus macaques - each with implants in M1 consisting of two 64-electrode arrays, when they performed reach tasks with their right upper limbs constrained by a 2D exoskeletal robot. Given that single-trial high gamma amplitude envelopes were quite variable, we first denoised the single-trial envelopes using a contractive autoencoder [3], then smoothed and normalized the amplitude between 0 to 1 for each trial. The high gamma amplitude envelopes from different electrode locations tended to amplify around movement initiation at slightly different times; to quantify this difference, we computed the time at which the envelope on each electrode crossed a unified threshold. We then performed planar regression of the threshold-crossing times to characterize the spatiotemporal patterns, and computed the propagation orientation for each trial. We found that for both monkeys there existed salient propagating orientations for each array. Furthermore, for both monkeys, the exact orientation of this propagating pattern varied according to reach directions, indicating that the spatio-temporal patterns might encode movement-specific information. Next, we plan to explore whether and how these patterns might aid in the execution of movements. [1] Balasubramanian et al(2020). Propagating motor cortical dynamics facilitate movement initiation. *Neuron*, 106(3), 526-536. [2] Zhuang et al(2010). Decoding 3-D reach and

grasp kinematics from high-frequency local field potentials in primate primary motor cortex. *IEEE Transactions on Biomedical Engineering*, *57*(7), 1774-1784. [3] Rifai et al(2011). Contractive auto-encoders: Explicit invariance during feature extraction. In Icml.

Disclosures: W. Liang: None. **K. Balasubramanian:** None. **V. Papadourakis:** None. **N.G. Hatsopoulos:** None.

Poster

P542. Vocal and Orofacial Motor Control: Model Organisms

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Program #/Poster #: P542.01

Topic: E.04. Voluntary Movements

Support: NS107466
MH117778

Title: Vocal specific premotor neurons in the mouse brainstem

Authors: *J. PARK[1], F. WANG[3,2];
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Abstract: Vocalization is a critical orofacial behavior for communication in many species. How the brain precisely controls the activity of distinct muscles to produce vocalization is largely unknown. The brainstem contains many orofacial-pharyngeal motoneurons and their premotor neurons. Due to technical challenges, previously vocalization-specific premotor neurons in the brainstem have not been clearly identified, and thus target-specific functional manipulation has not been possible. Here, we map premotor neurons of laryngeal muscles, and identify a vocal-specific premotor population in the brainstem of adult mice. Utilizing a three-step monosynaptic rabies virus transsynaptic tracing approach, we found that the laryngeal premotor neurons were distributed in the brainstem with three major clusters in: the Retroambiguus Nucleus (RAm), the PreBotzinger Complex, and the Nucleus Tractus Solitarius. Furthermore, c-fos, an immediate early gene is strongly induced in the RAm area by vocalizing behaviors. We therefore employed an activity-dependent tagging technique, called Capturing Activated Neuronal Ensemble (CANE) to tag vocalization-activated c-fos-positive RAm neurons (RAm^{VOC} neurons hereafter), whose location overlaps with that observed for laryngeal premotor neurons in the RAm. Tracing axonal projections in the brainstem co-stained with a motoneuron marker, ChAT revealed that the RAm^{VOC} neurons were indeed premotor neurons extensively innervating laryngeal motoneurons. We next expressed tetanus light chain in RAm^{VOC} neurons using CANE. Interestingly, bilateral inhibition of the RAm^{VOC} neurons completely abolished vocalizations,

including both ultrasonic vocalizations and audible stress-response squeaks. Taken together, RAm^{VOC} neurons are premotor neurons essential for vocalization.

Disclosures: J. Park: None. **F. Wang:** None.

Poster

P542. Vocal and Orofacial Motor Control: Model Organisms

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Program #/Poster #: P542.02

Topic: E.04. Voluntary Movements

Support: NIH Grant 5R01DC018691-02
Howard Hughes Medical Institute
Keck Foundation

Title: Investigating genetic regulation of cortical vocal motor circuits in mice

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Abstract: Vocal learning is the ability to learn and modify vocalizations, which is an essential trait for spoken language in humans. Vocal learning in humans as well as in songbirds is thought to be facilitated by a direct projection between primary motor cortex (MOP) and neurons in the brainstem involved in speech. Mice also show rudimentary signs of vocal learning circuitry and behavior, as they are able to modulate their high-frequency vocalizations and they possess a direct, but sparse corticomotoneuronal projection. Little is known about the evolution of these projections from MOP to brainstem, specifically the genetic pathways involved in the development of these circuits. Comparative genetic studies between human and songbird vocal areas have found a number of convergently expressed genes, including axonal guidance genes. Through manipulation of these genes in mouse MOP, we can elucidate how axon guidance cues regulate projections from MOP to brainstem related to vocalization. We generated transgenic mice with knockdown of PlxnA1 and Slit1 in layer 5 neurons of putative mouse laryngeal motor cortex, to mimic expression in vocal learners. Preliminary experiments suggest that these mice have greater axonal connectivity from layer 5 neurons to the vocal motor neurons of the brainstem, nucleus ambiguous. These studies contribute to a greater understanding of the mechanisms and evolution of vocal learning in advanced vocal learners.

Disclosures: L.C. Kuper: None. **L. Boyd:** None. **E.D. Jarvis:** None.

Poster

P542. Vocal and Orofacial Motor Control: Model Organisms

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Topic: E.04. Voluntary Movements

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Title: Locating larynx motor representation in fruit bats (Rousettus aegyptiacus) with intracortical microstimulation (ICMS) optimized for detection of weak electromyographic (EMG) signals

Authors: *M. HAFEZI[1], A. C. HALLEY[2], J. HYSTAD[1], T. A. SCHMID[4], M. M. YARTSEV[4,5], L. A. KRUBITZER[2,3], D. F. COOKE[1];
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Abstract: Bats exhibit several traits associated with human communication that are rare among mammals, such as complex social communication and potentially vocal learning. Here, we investigate the cortical representation of the bat larynx as a model for the motor control of complex vocal behaviors. We applied ICMS in anesthetized Egyptian fruit bats to evoke muscle contractions and determine which areas of the neocortex control muscles of the larynx involved in social vocalization. We tested for the presence of a larynx representation in M1, S1, and adjacent cortex by recording EMG activity from the cricothyroid muscle during motor stimulation. In order to correct for EMG artifacts introduced by the ICMS signal itself, we varied ICMS circuitry (e.g. return lead placement, current sense resistor) to minimize contamination and reduce the need for “blanking” techniques, which can lead to signal loss. These corrections reduced the artifact by ~90% and increased the EMG signal-to-noise ratio, providing a robust laryngeal signal. Here we report that muscles of the larynx in a single case study were activated by ICMS at two rostromedial sites in M1. Cricothyroid activation occurred with distinct latencies for the two sites (20 vs 190 ms). Ongoing experiments will continue to survey a large region of cortex while also focusing on denser mapping of this putative larynx region, with the goal of understanding whether latency differences reflect modules within M1 or distinct cortical fields, perhaps with different roles in the control of vocal behavior. A more complete understanding of

the motor cortical representation in bats, including that of a potential laryngeal representation, could help test hypotheses about the neural mechanisms that support vocal communication.

Disclosures: M. Hafezi: None. **A.C. Halley:** None. **J. Hystad:** None. **T.A. Schmid:** None. **M.M. Yartsev:** None. **L.A. Krubitzer:** None. **D.F. Cooke:** None.

Poster

P542. Vocal and Orofacial Motor Control: Model Organisms

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Topic: E.04. Voluntary Movements

Support: NIH Grant 5R01DC018691-02
Howard Hughes Medical Intitute
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Title: Stimulation of Mouse Primary Motor Cortex Activates Intrinsic and Extrinsic Vocal Muscles

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Abstract: Vocalizations are a dynamic and complex behavior that can be innate or learned. Vocal learners, animals that learn to imitate or modify vocalizations, use top-down input from the forebrain to provide fine motor control over vocal musculature. These forebrain circuits coordinate sub-cortical networks during and after learning to precisely execute these learned behaviors. Currently, we do not have genetically tractable mammalian models for speech and vocal learning. However, behavioral and anatomical findings from our lab and others' show that the ultrasonic song system of mice exhibits some rudimentary features of vocal systems found in advanced vocal learners, including multisyllabic structure that varies with social context, and a sparse but direct input to laryngeal motor neurons in the brainstem from a small pool of neurons in the primary motor cortex (M1). This population of laryngeal-projecting M1 neurons were termed the laryngeal motor cortex (LMC). Work from others has also shown that the orofacial motor cortex (OFC), located anterolaterally in the motor cortex, is involved in controlling vocal behavior by influencing the digastric muscle. Using intracortical microstimulation with paired electromyography (ICMS/EMG), we tested whether electrically exciting the previously identified LMC and OFC regions could influence vocal muscles. We recorded EMGs from the cricothyroid muscle, an intrinsic laryngeal muscle, and the digastric muscle, related to jaw opening. Our results confirm previous studies showing that stimulating the OFC can induce digastric muscle contractions. In addition, we found that stimulating the LMC induces muscle contractions in the larynx. By measuring the latency of the muscle response after ICMS, we find

that both regions have very short response times. The OFC in particular produced consistently faster responses (5-10 ms) than the LMC (10-20 ms). The latencies from the OFC are in line with those found in monosynaptic circuits, suggesting this region may be more involved in fine motor control of vocal behaviors than had been previously considered. We suggest that the mouse LMC could be the precursor of human dorsal LMC (dLMC), and the mouse OFC the precursor of human ventral LMC (vLMC). Overall, these results represent the first time that stimulation of the primary motor cortex in a mammal, other than humans, has resulted in laryngeal muscle contractions.

Disclosures: C.D.M. Vargas: None. **E.N. Waidmann:** None. **E.D. Jarvis:** None.

Poster

P542. Vocal and Orofacial Motor Control: Model Organisms

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Title: Characterization of Pre-motor Region Contributions to the Productions of Call

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Abstract: Songbirds are a powerful model for studying the acquisition and production of complex learned vocal behavior. Zebra Finches in particular have been heavily studied for their stereotyped motif which is a major component of their song. However, the syllables of the motif are only a small portion of the zebra finch's vocal repertoire. The majority of their vocal behavior consists of vocalizations collectively referred to as 'calls'. Calls refer to all vocalizations that are not the introductory note or part of the stereotyped motif; some are learned and others are not. While previous work has found that neural activity in pre-motor nuclei (i.e. HVC and RA) encodes information regarding which syllable the bird will sing, few studies focus on elucidating what activity, if any, relates to call production. Here we report novel spectral features of neural activity that correlate with call identity in a premotor nuclei. We applied

unsupervised dimensionality reduction techniques to map spectrograms of manually segmented Calls to a lower dimensional representation. The spectrograms of the calls were then manually clustered and annotated based upon this representation. The neural activity recorded during each call was then analyzed using methods previously used to study motif syllable production (Brown et al. 2021). We report finding phase preference to motor onset of calls similar to that reported to syllable production in HVC of songbirds. Specifically, we find statistically significant Inter-Trial Phase Coherence (ITPC) in Local Field Potentials (LFP) across multiple types of calls in frequencies ranging from 2-50 Hz. Our results give preliminary evidence that the pre-motor nuclei of the song system play a role in the production of vocal units other than the syllables of the stereotyped motif. These results elucidate the relationship between pre-motor nuclei in the song system to call production and help clarify the function of these nuclei in regards to the generation of vocal behavior.

Disclosures: D. Brown: None. **A. Srinivasan:** None. **P.M. Tostado:** None. **J.I. Chavez:** None. **D.H. Nguyen:** None. **A. Kadwory:** None. **E.M. Arneodo:** None. **T. Gentner:** None. **V. Gilja:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); V.G. holds shares in Neuralink, Corp., and Paradromics, Inc. These organizations had no role in study design, data collection and analysis, decision to publish, or preparation of the abstract.. F. Consulting Fees (e.g., advisory boards); V.G. currently consults for Paradromics, Inc. They had no role in study design, data collection and analysis, decision to publish, or preparation of the abstract..

Poster

P542. Vocal and Orofacial Motor Control: Model Organisms

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Program #/Poster #: P542.06

Topic: E.04. Voluntary Movements

Support: BWF CASI
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Title: Information processing by neuronal networks in singing and sleeping zebra finches.

Authors: *W. G. GONZALEZ, F. LANFRANCHI, C. LOIS;
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Abstract: How is information processed by neurons during performances of a complex motor behavior and how does sleep affect maintenance of such behavior? To address these questions we simultaneously recorded the activity from neurons in multiple brain areas of zebra finches. Songbirds are a well-established animal model of vocal learning but few studies have studied the mechanism by which information transfer across brain areas controls singing behavior. Using

high density extracellular probes (Neuropixels) we recorded the activity from hundreds of neurons across two premotor nuclei (HVC and RA) as well as from auditory brain areas in singing and sleeping zebra finches. Neurons in these brain areas are known to control singing behavior and have been proposed to replay the song during sleep. By monitoring the online and offline communication between neurons across these brain areas we identify mechanisms by which premotor neurons control, monitor, and rehearse a complex learned behavior. During performance of the song, slow oscillation (1 - 5 Hz) in HVC and RA mark the start and end of a song while packets of high frequency oscillations (20-100 Hz) relay song specific information. Production of an abnormal song is preceded by an increase in synchronous activity in HVC and RA. Simultaneous bilateral recordings during sleep identifies offline periods during which partial representations of the song are asynchronously reactivated across hemispheres. These results highlight a hierarchical organization of neuronal activity during performance of a complex motor behavior and identify the presence of mechanism that asynchronously modulate neuronal activity across hemispheres during sleep.

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Poster

P542. Vocal and Orofacial Motor Control: Model Organisms

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Mizzou Advantage

Title: Experimental method for longitudinal investigations of behavioral adaptations in individualized orofacial movements of rats using XROMM

Authors: *K. TAKAHASHI[1], C. HAXTON[1], T. E. LEVER[2], B. SAGL[3], L. AVIVI-ARBER[4], S. FELS[5], I. STAVNESS[6];
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Abstract: It has been shown in rat models that intraoral manipulations (e.g., tooth extraction or dental implants) induce neuroplasticity in the orofacial sensorimotor cortex and corresponding

morphological and histochemical changes in cranial muscle tissue, and that neurological disease models (e.g., Parkinson's) exhibit oropharyngeal dysfunction. However, data is lacking relative to high fidelity kinematic and behavioral changes in orofacial functions over time. Moreover, a major challenge of using rats for long-term kinematic studies is that their musculoskeletal system grows over time. Here, we describe our initial efforts in establishing robust methodology for conducting chronic orofacial kinematic studies in rats. We combined a high-speed biplane x-ray reconstruction of moving morphology (XROMM) system to capture radiopaque marker movements over time, obtained in-vivo CT scans to capture skeletal morphological changes over time, and combined these two data sources to produce high throughput orofacial kinematics through an individually customizable simulation model. To achieve this, we implanted radiopaque tantalum markers within the cranium, right and left hemimandibles, and the tongue of 4 adult rats (3 females and 1 male) that were at least 12-week old and weighed >250 grams. To dampen growth, animals underwent caloric restriction to reduce the number of the CT scans. XROMM sessions took place twice a week for at least a month to ensure at least 5 mins of self-feeding was recorded for each animal while eating radiopaque kibble. XROMM videos were sampled at 400 FPS and digitized by XMALab. X-ray (90 kV and 25/20 mA) was emitted only when animals were engaged in feeding to minimize the risk of radiation-induced symptoms. In-vivo CT was obtained every 3-4 weeks to capture morphological changes over time. The ArtiSynth modeling toolkit was used to develop a rat orofacial simulation model to compute the joint angle and 3D translation of each hemimandible and tongue movements from the marker locations in the cranium coordinate system. Results showed that right/left hemimandible movements were somewhat independent, but all animals showed the highest normalized standard deviation of translation movements along the anterior-posterior axis. Additionally, two animals exhibited significant differences in gape duration across days despite constant kibble consistency. Once optimized, we envision our new experimental workflow from marker implant surgery to kinematic analysis will open a gateway for longitudinal high throughput studies of orofacial function in rats while controlling for individual morphological changes due to skeletal growth.

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Poster

P542. Vocal and Orofacial Motor Control: Model Organisms

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Title: Short term behavioral adaptations in gape cycle patterns due to tooth extraction

Authors: *C. HAXTON[1], K. TAKAHASHI[1], N. SHIRAISHI[2], T. E. LEVER[3], L. AVIVI-ARBER[4], A. ZIMMERMAN[4], N. KATARIA[4], B. SAGL[5], C. ROSS[1], M. INOUE[6]; [1]Organismal Biol. and Anat., Univ. of Chicago, Chicago, IL; [2]Div. of Advanced Prosthetic Dent., Tohoku Univ., Sendai, Japan; [3]Otolaryngology - Head and Neck Surgery, Univ. of Missouri Columbia, Columbia, MO; [4]Univ. of Toronto Dent., Toronto, ON, Canada; [5]Univ. Clin. Dent., Med. Univ. of Vienna, Vienna, Austria; [6]Niigata Univ., Niigata, Japan

Abstract: It has been shown in rodents that intraoral manipulations (i.e., tooth extraction or dental implants) induce neuroplasticity in the orofacial sensorimotor cortex as well as morphological and histochemical changes in cranial muscle tissue. However, little is known about how fast and what kind of behavioral changes may occur. To answer these questions, we implanted radiopaque markers (0.5mm diameter) within the cranium, right and left hemimandibles, and tongue of two adult female rats that were at least 8 weeks old and weighed 200 grams. We used a high-speed biplane x-ray reconstruction of moving morphology (XROMM) system to capture marker movements, which were projected onto a cranium coordinate based on in-vivo CT images of the skull markers. This produced 3D movements of the hemimandibles and tongue relative to the cranium. Next, the three right maxillary molars were luxated under aseptic conditions, consistent with our previously published protocols. XROMM data during unconstrained feeding of radiopaque kibble were collected at 250 FPS prior to molar extraction, serving as a baseline, as well as 1, 2, 3, 4, 7, & 14 days post-extraction to characterize fast behavioral adaptations for chewing and swallowing. XROMM data were digitized by XMALab and the hemimandible movements, gape angle, and 3D translations were computed. There are two well-defined jaw gape cycles: rhythmic chewing (RC) and swallowing (S). For cycle type assignment, each video was examined to check if a gape cycle in a feeding sequence was rhythmic, then further checked for swallowing. Cycles containing swallowing events were labeled as S, and those with only rhythmic chewing were labeled as RC. We then analyzed how RC and S cycle frequency changed across days. During the pre-tooth extraction period, median gape frequencies were significantly higher ($p<0.05$) for RC cycle (7.14 Hz) than S cycle (6.02 Hz). Both RC and S cycles were significantly slower on Day 1 (RC: 5.99 Hz vs S: 3.72 Hz) and the variance increased significantly compared to baseline (RC: 2.65 times vs S: 2.45 times). From Day 2 to Day 7, both RC and S cycle frequencies became faster; however, RC cycle variance decreased, while S did not change ($p>0.05$) after Day 3. Our results show that, in addition to the previously reported slow adaptations in the central and peripheral nervous systems, molar tooth extraction induces rapid behavioral changes with different RC and S gape cycle statistics. Our findings suggest there may be corresponding fast neural adaptations and tissue regeneration taking place that have not been rigorously investigated. Furthermore, dental integrity may be more important for swallowing than generally thought.

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Poster

P542. Vocal and Orofacial Motor Control: Model Organisms

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Title: Specializations of tongue motor cortex for lingual echolocation in the Egyptian fruit bat (Rousettus aegyptiacus)

Authors: *A. C. HALLEY[1], M. M. YARTSEV[2], L. A. KRUBITZER[3];
[1]Univ. of California, Davis, Davis, CA; [2]Bioengineering, Univ. of California Berkeley, Berkeley, CA; [3]UC Davis, Davis, CA

Abstract: The Egyptian fruit bat (*Rousettus aegyptiacus*) is one of the only megabats to echolocate, and is rare among bats in its use of tongue-clicks to generate active sonar. Remarkably, *Rousettus* produces directional sonar without moving its head or jaw, instead altering the frequency and direction of sonar clicks by manipulating its tongue. In recent mapping studies using intracortical microstimulation (ICMS), we found that representations of the tongue are enlarged in motor regions of the neocortex in *Rousettus*. However, little is known about how the precise tongue movements involved in echolocation are generated in the neocortex. To better characterize this specialized motor behavior, we used long-train ICMS to elicit movements from areas of the neocortex involved in tongue movement (e.g. M1, S1). We found that movements of tongue subregions (e.g. distal vs. proximal) as well as distinct movements types (e.g. extension) are represented regularly across areas of cortex involved in generating movement. This kind of discrete representation of tongue subregions and movement types in the neocortex is rare among mammals, and suggests that motor systems in *Rousettus* have evolved for specialized tongue control in order to produce their novel form of lingual echolocation.

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

Poster

P544. Neurophysiology: Non-Invasive Techniques II

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Program #/Poster #: P544.01

Topic: E.05. Brain-Machine Interface

Support: NSF Grant 1849213

Title: Discrimination of different levels of finger extension from the electroencephalogram

Authors: M. BATES, C. HADDIX, *S. SUNDERAM;
Biomed. Engin., Univ. of Kentucky, Lexington, KY

Abstract: Assistive technologies such as brain-computer interfaces (BCIs) offer individuals with central or peripheral neural injuries the means to interact with external devices. However, the reliance on EEG for convenient access to the brain limits the ability to accurately quantify control signals associated with fine motor control, especially in victims of stroke, who may have cortical lesions. Here we test the feasibility of modeling gradations in motor effort associated with a finger extension task using EEG measurements. Following an IRB-approved protocol, we acquired scalp EEG in three healthy human subjects. Instructions were given to extend their fingers outward to one of three levels in response to a visual cue: Low, Medium, and High, as well as a fourth control condition, 'No-Go', in which no movement is expected. The subject's hand movement and muscle activity (extensor digitorum communis) were monitored using a commercial motion capture glove and bipolar EMG, respectively. The glove recorded each phalange's position in Cartesian space, which allowed us to track extension of each digit relative to the wrist. We focused on the index, middle, and ring finger alone since they are similar in length to minimize error, and averaged the smoothed measurements from these three fingers to produce a scalar displacement in degrees. EEG was simultaneously recorded over 32 scalp locations and the mean-squared power in a moving window estimated in the 8 - 30 Hz band during each cue relative to a reference period before cue onset. A quadratic discriminant classifier was constructed from training samples of this feature and the accuracy of prediction of test samples was assessed using a four-fold cross-validation scheme. The mean classification accuracy was 57% over the four levels of extension, much greater than the chance level of 25%. Confusion between low and medium extension was the major source of error. By comparison, using a similar approach, measures of EMG power and finger displacement gave mean accuracies of 53% and 47%, respectively, an indication that variability in the subject's performance of the task may have contributed at least in part to the error. Our findings show that the EEG can predict effort associated with gradations in finger extension. This offers BCIs a measure of fine control for interactive rehabilitation protocols. Future work will focus on closing

the loop using these distinguishable signals to provide real-time feedback, which can be incorporated into existing regimens to promote functional recovery.

Disclosures: M. Bates: None. **C. Haddix:** None. **S. Sunderam:** None.

Poster

P544. Neurophysiology: Non-Invasive Techniques II

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Program #/Poster #: P544.02

Topic: E.05. Brain-Machine Interface

Support: NSF award 1539068
NSF award 1430833

Title: Quicker Proficiency in Imagined Cursor Tasks with Tripolar Concentric Ring Electrodes

Authors: *W. G. BESIO[1,2], P. G. ADEGBOYEGA[1];
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Abstract: Brain-computer interfaces (BCIs) allow the translation of brain signals into device control signals. Tripolar concentric ring electrodes (TCREs) for electroencephalography (tEEG) were first reported by Besio (2006). The tEEG has significantly higher spatial resolution and signal to noise ratio (SNR) than EEG. The tEEG was also shown to have significantly less mutual information than EEG. The tEEG has previously been shown to improve BCI accuracy in offline classification and for real-time center-out cursor control compared to EEG. We have also shown that the outer ring of the TCRE produces an equivalent signal to EEG (eEEG) and TCREs can therefore record EEG and tEEG from the same sensor concurrently. In this study we compared whether naïve BCI users could become proficient with less runs using either tEEG or EEG.
We evaluated how quickly naïve BCI users could achieve proficiency with conventional EEG or tEEG recorded from TCREs. The study was approved by the URI IRB. We tested 12 healthy BCI naive subjects, randomly assigned, six each, to standard EEG or tEEG, with their goal to, "hit", sixty percent of the targets in a single "run" of an imagined movement cursor task. The TCREs were placed at CP3, C3, C1, Cz, C2, C4, CP4, of the 10-20 International Electrode positions, as well as a conventional disc electrode on each mastoid process as the ground and reference. The skin to electrode impedances were kept below 10 kΩ. Each participant completed a short training/ calibration session in the form of a stimulus presentation to obtain data for the individual classifiers. Participants were asked to remain still and imagine motor imagery tasks as arrows pointing left or right appeared on a monitor directly in front of them. Offline r^2 analysis using BCI2000 was performed to determine the locations and frequencies used for the real-time

classifiers. Participants were blinded to the type of electrodes signals they were using. Five out of six participants in the EEG group were unable to reach sixty-percent proficiency, even after 14 runs. Conversely, five of six tEEG participants reached sixty-percent proficiency, and the sixth reached fifty-five percent proficiency, higher than most of the EEG participants. The results show that significantly more participants were able to reach >= to sixty-percent proficiency within less runs using tEEG compared to EEG. There was a significant difference in the number of proficient participants (Mann Whitney unpaired test with correction $p = 0.017$). Once a participant reached sixty-percent proficiency they did not perform any further runs. The tEEG participants likely would have had even higher hits if they continued beyond reaching sixty-percent.

Disclosures: W.G. Besio: A. Employment/Salary (full or part-time):; CREmedical Corp.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); CREmedical Corp..

Poster

P544. Neurophysiology: Non-Invasive Techniques II

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Topic: E.05. Brain-Machine Interface

Support: NIH NS102259

Title: Non-invasive hands-free control of a robotic arm

Authors: *D. E. G. SHEETS, B. A. HASSE, D. M. GIN, Y. FAHMY, A. J. FUGLEVAND; Physiol. and Neurosci., Univ. of Arizona, Tucson, AZ

Abstract: Simple tasks such as using a computer, feeding oneself, personal hygiene, and grabbing objects are impossible for high level tetraplegics without assistance. Most of these individuals, however, retain the ability to voluntarily move their head, eyes, and tongue and can speak. It seems reasonable to hypothesize that signals derived non-invasively from these actions could be used to accurately control movements of a robotic limb or a paralyzed upper limb using functional electrical stimulation. The goal of this project, therefore, was to evaluate various hands-free methods to position the endpoint of a robotic arm to different locations. Healthy adult human subjects participated in each of five experimental sessions for this study. In four of the sessions, subjects used one of four input modalities (head, tongue, eyes, and for comparison, movements of their own hand) to move the tip of a robot arm to different targets. Within each session, two types of continuous control were enacted: position and velocity. In position control, the position of the input device was mapped to the position of an effector - like the position of a computer mouse dictating cursor position on a screen. In velocity control, the position of the

input device controlled the velocity of the effector - like a gas pedal in a car. In a fifth session, subjects used discrete voice commands to control movements of the robotic arm. When using the head, tongue, or hand, signals from small 6 degree-of-freedom movement sensors attached to those body parts were used as inputs to the robot arm. For the eyes, measured pupil movements were used to estimate gaze location that served as the input to the robot arm. Eye movements were only used in the context of position control. Once instrumented and having practiced, subjects performed a set of 72 trials, involving 6 reaches to 12 different targets for each control system. Physical targets were wooden rings mounted on supports of different heights. For a successful reach, the tip of a pointer attached to the robot arm needed to be situated and held for 1 s within a virtual 5-cm diameter sphere centered in the middle of the wooden ring. Movement time, path length, and throughput were calculated for each reach. Overall, head control was just as effective in controlling robot-arm reaches as that using the hand. Position control outperformed velocity control in all modalities except for that involving the tongue. These findings provide critical insight into the utility of various non-invasive methods to control an assistive robot arm and should improve the ability individuals with high level paralysis to interact with their environment.

Disclosures: D.E.G. Sheets: None. **B.A. Hasse:** None. **D.M. Gin:** None. **Y. Fahmy:** None. **A.J. Fuglevand:** None.

Poster

P544. Neurophysiology: Non-Invasive Techniques II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P544.04

Topic: E.05. Brain-Machine Interface

Support: DARPA N65236-19-C-8017

Title: Steerable localized electrical stimulation using high density subdermal electrode arrays in rodents

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Abstract: Objective: Transcranial stimulation using large current-injection electrodes has shown effects in improving learning,memory, movement disorders, pain, etc. The large size of electrodes results in a diffuse electric field in the brain, preventing targeted localized neural stimulation. Recent efforts in using high-density transcranial direct current stimulation (HD-TDCS) have resulted in improved outcomes and reduced side-effects. However, typical HD-TDCS uses electrode distances of several cm in humans and above 5 mm in rodents. Increased

electrode density can improve the field focus and enable beam steering to actively target different regions within the brain.
Methods: Here, we discuss a method for high resolution steerable electrical stimulation using a 6x4 array of high-density electrodes (0.4 mm diameter electrodes in a 1 mm grid) implemented using flexible PCB technology. The electrode grid is placed directly over the skull of C57BL6 mice (n=7, aged 4 to 6 weeks). Multiple current-injection patterns are generated by selectively activating subsets of electrodes. The current is injected via 2x2 active electrodes, positioned in one of three locations, located mediolaterally at -2,0, and +2 mm from Bregma. The spatial extent of the electric field within the tissue is affected by changing the distance between the active electrodes and four return electrodes (one at each corner) from 1 to 3 mm. Evoked responses are measured using electromyography (EMG) of limbs and video recording of whisker movements. For EMG, selectivity is assessed by measuring the amplitude of the motor evoked potential (MEP) in the different muscles. Intracortical microstimulation (ICMS) using implanted metal electrodes is used as a ground truth to validate the response to electrical stimulation.
Results: The results from the current study showed preferential contralateral response by steering active electrodes on either side of the skull as evidenced by higher MEP amplitude and whisker movement on the contralateral side. An equal bilateral response was observed when the active electrodes were centered on Bregma. Selectivity can be enhanced by tuning the pattern with more focused current fields, at the cost of higher current injection. Interestingly, whisker movement changes from contralateral to ipsilateral when the more focused patterns are used.
Conclusion: Focusing of transcranial currents using high density electrode arrays allows selective motor responses in mice. High density arrays enable adjusting the location of the stimulation as well as the extent of the stimulated region. This could potentially open new avenues for BCI applications.

Disclosures: V. Jain: None. **M. Forssell:** None. **D. Tansel:** None. **C. Goswami:** None. **G. Fedder:** None. **P. Grover:** None. **M. Chamanzar:** None.

Poster

P544. Neurophysiology: Non-Invasive Techniques II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P544.05

Topic: E.05. Brain-Machine Interface

Title: Validation of an electrical head phantom model for EEG motion artifacts susceptibility

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Abstract: In recent years there have been many new approaches developed to improve the convenience and quality of data extracted from EEG collected in real-world environments. These range from new materials for “dry” or quick-application sensors to multi-layer sensing, or post-collection de-noising techniques. In most cases these target the removal of noise from motion or other non-brain sources. However, a continual challenge is that it is extremely difficult to prove or validate the efficacy of these approaches, given the lack of a true ground-truth comparative signal (we don’t know what the original true signal looks like). One method to address this problem is the use of an EEG “phantom” which provides a known-quantity signal propagated through a simulated conductive head and scalp to use in comparing new methods. Previous efforts have suggested use of ballistics gelatin as a substrate, but it’s performance as a surrogate has not been fully validated. Here we describe the process of validating a phantom head formulation for re-creating motion artifact susceptibility associated with EEG electrodes or similar DAQ components. EEG data were collected from human volunteers walking on a treadmill simultaneous with optical motion capture (MoCap) while wearing a Biosemi 64 channel cap. Phantom head models with 0.5cm scalp thickness over an ABS inner continuous hollow skull were fabricated based on designs from Open EEG Phantom (https://osf.io/qrka2/) using ballistics gelatin (BG) (200g/L) or agarose (20 g/L, 0.0125% NaCl) outer layers. Phantoms were affixed to a 6dof Heaxpod to re-create each human’s motion based on profiles from that individual’s MoCap while wearing the identical Biosemi cap. RMS amplitude of the signal for different walking speeds was then compared against human data for both materials. Results show that BG does not re-create average artifact amplitude or the step-to-step variability of human data. Data collected on an agarose phantom more accurately recreates mean power and variability across gait cycles of human subject movement much better, supporting use as a phantom skin material. Notably within-subject, cross-trial variance remains substantially higher for native human data, likely reflecting physical components not re-created in the phantom (e.g. hair, jerk movements).
Reference: “Open EEG Phantom” found on Open Science Framework, https://osf.io/qrka2/

Disclosures: M. Nonte: None. **J.C. Bradford:** None. **A. McGough:** None. **W. Hairston:** None.

Poster

P544. Neurophysiology: Non-Invasive Techniques II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P544.06

Topic: E.05. Brain-Machine Interface

Title: “Human as a Phantom” - a repeatable method for validating biosignal sensing devices using a human volunteer testbed

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Abstract: There is an evolving interest in capturing Electroencephalogram (EEG) in ecologically valid environments: while subjects are moving or are performing tasks in the field. This introduces large-amplitude nonstationary noise into an already small, nonstationary signal. Given we are attempting to measure an unknown, nonstationary signal with unknown noise, fully understanding this noise to create an appropriate filter is an arduous task. Recent work has focused on making better electrodes or other data acquisition (DAQ) devices, but validating the signal efficacy of new sensors or DAQ techniques is difficult with only human subject comparisons, which are variable and inconsistent over time. Instead an alternate approach is to use a "phantom" device to provide a consistent testbed and ground truth signal for analysis. Some research groups have developed gel-based phantom heads for independently testing and removing this noise; these can reproduce properties of the skin-electrode interface relevant to noise and can reproduce limited realistic human movement. These phantoms lack several important physical features (e.g. hair) that introduce inter-subject variability and require better range of motion to fully recreate human movement.
Here we introduce a method for examining and evaluating EEG and other biosignal capture equipment using a live human as the testbed, providing the realism of skin, hair, or motion, but with a known-quantity ground truth signal. We use a voltage-controlled transcranial electrical stimulator (tES) attenuated to appropriate biosignal level to inject a test signal on the scalp of a human volunteer. By evaluating that signal, we are able to analyze time synchrony of various devices as well as noise inherent in the systems' sensing. This has the advantage of capturing the natural human-to-human variance in signal quality as well as the naturally evolving interface with human skin that is otherwise difficult to replicate. For this proof-of-concept, we compare two EEG systems (g.tec and OpenBCI) and two electrode types (traditional gel/AgCL and g.tec gold metal pin), as well as a Bioharness monitoring system through a short series of artefact-generating movements. We are able to accurately extract the original signal, better synchronize the systems, and determine the noise profile of each system. Future protocols could include miniaturized signal generators integrated with biocapture systems to better understand the noise associated with sensing in real-world environments.

Disclosures: C. Sinks: None. **M. Nonte:** None. **W. Hairston:** None.

Poster

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Program #/Poster #: P544.07

Topic: E.05. Brain-Machine Interface

Support: NIH P41EB018783-07 (J.R.Wolpaw) Stratton VA Medical Center R01HD062744

Title: Determining neural correlates of proprioception in order to improve robotic-assisted movement training (RAMT)

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Abstract: Robot-assisted movement training (RAMT) is increasingly popular in neurorehabilitation. However, its current benefits are limited and highly variable. Better RAMT protocols are needed. A recent study of hand function in people with stroke showed large finger proprioceptive error measured before RAMT predicted minimal benefit of subsequent RAMT (NNR 2017). Anatomical damage to the somatosensory network was better explained as treatment-related hand function gains than damage to the motor network (Neurology 2019). Better understanding of the neural correlates of proprioceptive error could lead to more effective RAMT protocols, such as protocols that blend RAMT with brain-computer interface training of those correlates. The previous study used a novel crisscross paradigm with a customized robotic exoskeleton. We add a concurrent EEG recording to this paradigm and describe our preliminary observation. Data was collected from five healthy people (4m/1f, 22-66yrs) with informed consent (all experiments approved by the Stratton IRB). The exoskeleton performed passive movements of the right index and middle fingers in a pre-programmed crisscross protocol. The fingers moved continuously between full extension and 60 deg flexion. 80-100 crossover trials with randomized inter-trial intervals were collected with vision occluded. The task was to press a button with the left index finger when the right index and middle fingers crossed over each other. Scalp EEG (32 channels) was recorded with reference to the right mastoid and digitized (256-Hz) (NCAN BCI2000 software; gUSB amplifiers (g.tec GmbH, Austria)). A MEMS inertial sensor measured the button press movement and robot position sensors recorded finger metacarpophalangeal (MCP) joint trajectories. Proprioception error was calculated as the difference in degrees of MCP joint rotation of the two fingers at the time of button press. Errors were categorized as small (<6deg) and large (>6deg). EEG was pre-processed and denoised with xDawn. Focusing on the sensorimotor electrodes contralateral to the robotically driven hand (C5 and C3), evoked responses for trials with small and large errors were averaged in the -2s to -1s window. Preliminary results (one subject) show the mean C3 and C5 ERP response was significantly more negative for the large error. Furthermore, a 32-channel logistic regression classification with a 10-fold stratified cross-validation showed error-related EEG feature classification, with precision more than chance level. This preliminary ERP outcome suggests the possibility of extracting a neural feature related to finger proprioceptive error with this paradigm.

Disclosures: C.M. Chaisson: None. **D.J. Reinkensmeyer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Hocoma, Flint. F. Consulting Fees (e.g., advisory boards); Flint. **T. Fake:** None. **J. Hill:** None. **T.M. Vaughan:** None. **D. Gupta:** None.

Poster

P544. Neurophysiology: Non-Invasive Techniques II

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Program #/Poster #: P544.08

Topic: E.05. Brain-Machine Interface

Support: NIH Grant P41 EB018783
Albany Stratton VA Medical Center

Title: Improving the performance of brain-computer interface-based color vision assessment using data interpolation methods

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Abstract: We have developed a BCI-based system for color-vision assessment that requires no behavioral responses. This system uses steady-state visual evoked potentials (SSVEPs) to identify metamers—light sources with different spectral distributions that appear to be the same color. Precise evaluation of the identified metamers requires dense sampling of the color space; each sample is obtained by adjusting the stimulus color and obtaining the SSVEP. Thus, dense sampling is time and labor intensive and may tire the participant.
One alternative to dense sampling of the color space is interpolation. Our metamer identification problem can be posed as a two-dimensional search problem; the system searches for the combination of a dichromatic (red+green) test source that appears identical to a monochromatic (amber) reference source of a fixed luminance. To evaluate the effectiveness of interpolation, we compared 5 interpolation methods to the dense sampling.
In a study with 5 healthy participants (1 male; ages 21-67) with normal color vision, we recorded EEG from 16 locations using BCI2000. Each participant completed 4 identical sessions of data collection. Each session included 168 trials; each trial sampled a different location in the dichromatic color space. During each trial, the participant looked at a stimulus that alternated between the two sources at 10 Hz. We used canonical correlation analysis (CCA) to extract SSVEPs. The Stratton IRB approved this study.
We averaged the CCA coefficients from all 4 sessions to obtain the ground truth for each

participant. We then computed the ratio of the interpolation error to the measurement error for each interpolation method. The number and order of interpolated values were picked randomly (we used 750 random selections). The average error ratio for the Gaussian interpolation was 1.52±0.50, lower than 1.55±0.59 for B-spline ($p=0.094$), 1.69±0.24 for linear ($p<0.001$), 2.18±0.30 for cubic ($p<0.001$), and 2.71±0.58 ($p<0.001$) for nearest-neighbor interpolation. On average, Gaussian interpolation had the smallest error. Additionally, for 2 participants, the error ratio of the Gaussian interpolation was <1 when <40 values were interpolated, indicating that the interpolation error was less than the measurement error.
In sum, these results suggest that interpolation is an effective alternative to densely sampling data for identifying metamers using SSVEPs. This should enhance our new system's value for evaluating people (e.g., children) in whom lengthy data collection is not practical.

Disclosures: H. Habibzadeh: None. **K.J. Long:** None. **A.E. Atkins:** None. **T.M. Vaughan:** None. **J.R. Wolpaw:** None. **D. Zois:** None. **J.J.S. Norton:** None.

Poster

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Program #/Poster #: P544.09

Topic: E.05. Brain-Machine Interface

Support: NIH Grant P41 EB018783
Albany Stratton VA Medical Center

Title: A BCI-based system for automatically assessing color vision

Authors: J. J. S. NORTON[1,2], **A. E. ATKINS**[3,1], H. HABIBZADEH[2,1], K. J. LONG[1], J. S. CARP[1], T. M. VAUGHAN[1], J. R. WOLPAW[1];
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Abstract: We describe a brain-computer interface(BCI)-based system for automatically assessing color vision that uses steady-state visual evoked potentials (SSVEPs) to identify *metamers*—light sources with different spectral distributions that appear to be the same color. Metamer identification is an established method for assessing color vision. It normally requires participation by the person under study. Recent research in our lab shows that a stimulus alternating between two metameric light sources—an amber monochromatic *reference source* and a red+green dichromatic *test source*—minimizes SSVEP size. Thus, by fixing the setting of the reference source and adjusting the settings of the test source, it is possible to identify metamers using SSVEPs, without the person's participation.

Here, we show that this BCI can identify metamers automatically. The system uses gradient descent to solve metamer identification as an optimization problem. First, the settings of the monochromatic source are fixed, and those of the dichromatic source are set to an initial estimate of the metamer. Then, SSVEPs elicited at nearby settings of the dichromatic source are measured. Based on these sizes, the system calculates a gradient and updates the dichromatic source settings of the initial estimate. The process repeats until the differences in sizes of the SSVEPs elicited near the new estimate of the metamer are below a criterion. The settings of the dichromatic source when the process stops define the metamer.
We tested our system by comparing its metamers to those identified behaviorally in 3 people with normal color vision (all experiments approved by the Stratton VA IRB). Each method matched the color of the dichromatic test source to the color of the monochromatic reference source at a luminance of 600 D/A units. The new system recorded EEG signals from 16 electrodes, elicited SSVEPs by alternating between the two sources at 10 Hz, and measured SSVEPs using canonical correlation analysis. Each person completed three runs with different initial settings of the dichromatic source (e.g., red,green=[0,0;100,0;0,100]). Averaged across participants the settings of the dichromatic source that were behaviorally- (red 149±14, green 49±1) and SSVEP-identified (red 124±7, green 63±11) as a metamer of the monochromatic source were similar. These results are an initial proof of concept that the new automated system can identify metamers.
An SSVEP-based BCI that can automatically assess color vision without active participation has numerous clinical, research, and industrial applications. Future work (e.g., optimizing search parameters) should further improve the new system.

Disclosures: J.J.S. Norton: None. **A.E. Atkins:** None. **H. Habibzadeh:** None. **K.J. Long:** None. **J.S. Carp:** None. **T.M. Vaughan:** None. **J.R. Wolpaw:** None.

Poster

P544. Neurophysiology: Non-Invasive Techniques II

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Program #/Poster #: P544.10

Topic: E.05. Brain-Machine Interface

Support: National Institute of Biomedical Imaging and Bioengineering of the NIH P41EB018783
Stratton VA Medical Center

Title: SSVEP-based color vision assessment: comparing data collection strategies

Authors: *K. J. LONG[1], H. HABIBZADEH[2,1], A. E. ATKINS[3,1], D.-S. ZOIS[2,1], J. J. S. NORTON[1,2], J. R. WOLPAW[1];
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Abstract: Color vision deficiencies (CVDs) affect >20% of people; they may impair school or work performance (*Jadhav et al. (2017)*). Our recent research (*Norton et al., under review*) shows that steady-state visual evoked potentials (SSVEPs) can be used to identify *metamers*—light sources with different spectral distributions that appear to be the same color, and that there are clear differences between the metamers of people with and without CVDs. Unlike other CVD detection methods, this method does not require participation; thus it may be useful in small children or those with cognitive deficits. This new method uses a stimulus alternating between a dichromatic test source and a fixed monochromatic reference source to elicit SSVEPs. Previously, we used a grid search of dichromatic source settings to find those that elicited the smallest SSVEP (i.e., the metamer of the reference source). This search sampled 72 dichromatic source settings, limiting the precision of metamer identification. More dense sampling of the grid requires a single much longer session (which may be impractical), or multiple sessions (which may introduce noise). Here, we compare two methods for sampling a dense grid (168 settings); one uses data from 1 session, the other uses data from 4 sessions over 4 days. Four healthy participants with normal color vision (1 male, age 21-67) completed 4 sessions. The Stratton IRB approved the study. Each session included: 4 runs, 42 trials/run, 7 s/trial. 16-channel EEG activity was recorded from the occipital region. SSVEPs were elicited by alternating between the monochromatic and dichromatic light sources at 10 Hz, and measured by canonical correlation analysis. We compared the two dense-sampling methods using universal kriging with an exponential trend model (*Murphy et al., PyKrige (2021)*). The average sum of absolute errors (defined as sum of the absolute differences between the interpolated and sampled canonical correlation coefficients) was 40.8±16.4 and 45.5±21.1 for the first and second sampling methods, respectively. There was a large effect size (*Cohen's d = 0.84*), but no significant difference (Student's paired t-test, $n = 4$, $p = 0.19$) between the two methods. 3 of 4 participants had more spatially continuous dense grids when data was collected in one session. The results suggest that constructing dense grids from data collected in one session reduces absolute error, and thus improves metamer detection. Alternatively, or in addition, interpolation might be used to improve the resolution of data obtained with a relatively coarse grid (*see adjacent Habibzadeh et al. abstract*).

Disclosures: K.J. Long: None. **H. Habibzadeh:** None. **A.E. Atkins:** None. **D. Zois:** None. **J.J.S. Norton:** None. **J.R. Wolpaw:** None.

Poster

P556. Neuroprosthetics: Control of Real and Artificial Arm, Hand, Other Grasping Devices II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P556.01

Topic: E.05. Brain-Machine Interface

Support: NIH R01 NS101013

Title: Resting-state electrocorticographic coherence changes from repeated brain-computer interface engagement

Authors: *S. S. SIVAKUMAR[1], S. A. ANAND[1], P. KARANDE[3], A. NOURMOHAMMADI[2], P. BRUNNER[2], J. C. WILLIAMS[4], E. C. LEUTHARDT[2], D. W. MORAN[1];
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Abstract: Epidural electrocorticographic (ECoG) electrodes serve as a minimally invasive tool to record cortical surface potentials (CSPs). The use of ECoG affords real-time, high-fidelity, chronic recording of CSPs at a high spatiotemporal resolution. In addition to lower frequency rhythms seen in EEG, ECoG is able to transduce high-frequency gamma band cortical activity (30-175 Hz). In a brain-computer interface (BCI), ECoG electrodes record modulation in brain activity. These electrophysiological features are decoded in real-time by a computer, with the output used to control an external device, such as a computer cursor.
In this work, four Rhesus macaques were trained to perform a two-dimensional cursor control task in virtual reality using a joystick. The primates had 32-channel platinum epidural ECoG arrays surgically implanted bilaterally over somatomotor cortex. They were then able to perform the cursor task via the BCI-control paradigm by modulating power across multiple frequency bands between 8 and 175 Hz. Baseline recordings from each BCI session were collected over six months of task performance. We hypothesized that chronic BCI-driven modulation in cortical power is associated with changes in resting-state magnitude-squared coherence of those CSPs that are decoded features in the BCI model. Here we show a rapid increase and a gradual return to baseline of task-free somatomotor CSP coherence over the time course of the BCI study period.

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Poster

P556. Neuroprosthetics: Control of Real and Artificial Arm, Hand, Other Grasping Devices II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P556.02

Topic: E.05. Brain-Machine Interface

Support: NIH 1DP5OD029571-01

Title: Improved Mirror-Therapy: Reanimation of Fine Motor Function Using Low-Cost Transcutaneous Electrical Nerve Stimulation Driven by Synchronized Bilateral Movements

Authors: *M. TROUT[1], T. S. DAVIS[2], J. A. GEORGE[1];
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Abstract: The long-term goal of this research is to develop a highly-specific and noninvasive reanimation system for the hand that can rehabilitate fine motor function for hemiparetic patients. Stroke is a leading cause of disability, and after a stroke, roughly 80% of patients experience weakness and lack of coordination on one side of the body known as hemiparesis. Mirror-therapy is a common rehabilitation intervention that uses a mirror to create illusory visual feedback of a paretic hand moving based on synchronized movements of the contralateral hand. Here, we introduce a system that leverages transcutaneous electrical nerve stimulation (TENS) of the arm nerves to reanimate a paretic hand in synchrony with the contralateral hand, thereby providing true visual feedback, as well proprioceptive and cutaneous feedback, to further engage neuroplasticity.

We developed a low-cost (~$60), high-voltage (+/- 300 V) Arduino-based stimulator capable of delivering arbitrary stimulation waveforms with up to 16 mA on three independent channels. Consistent with prior work, we found that TENS of the median, ulnar and radial nerves (applied to specific locations on the inner, upper arm) was able to produce repeatable, isolated finger flexion and extension across all digits, as well as functional combination movements (i.e., grasps).

A simple PID controller was used to drive TENS in order to match the grip forces exerted on bilateral load cells. A healthy participant was recruited to validate the preliminary efficacy of this system by relaxing one of their hands around a load cell to emulate a paretic hand and using their other hand to repeatedly squeeze a second load cell ten times. The stimulation-driven grasps yield 10.0 ± 1.32 N maximum contraction force without becoming uncomfortable, which was 29 ± 2.93% of the maximum-voluntary-contraction force from the contralateral hand. When working within the useable range of stimulation-driven forces (i.e., < 10 N), controlled stimulation matched the grip force between the two hands with 75.4 ± 0.97% accuracy (RMSE = 2.28 ± 0.22 N). For each grasp, there was a 113.6 ± 0.5 ms delay of stimulation-driven force onset and a 22.6 ± 3.71% difference in peak contraction force (7.47 ± 0.23 N in the “paretic” hand vs. 9.17 ± 0.65 N in the contralateral hand).

Further investigations will extend this work to acute and chronic stroke patients and measure short-term assistive benefits (e.g., grip force with and without stimulation), as well as long-term rehabilitative benefits (e.g., grip strength and hand dexterity before and after stimulation).

Disclosures: M. Trout: None. **T.S. Davis:** None. **J.A. George:** None.

Poster

P556. Neuroprosthetics: Control of Real and Artificial Arm, Hand, Other Grasping Devices II

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Program #/Poster #: P556.03

Topic: E.05. Brain-Machine Interface

Support: Kahn, Dan D. and Betty, Foundation AWD011321

Title: Error detection during the control of two finger-groups with invasive brain-machine interfaces

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Abstract: Summary: Brain-machine interfaces (BMIs) are prone to prediction errors. We have previously developed a method for detecting erroneous movements based on spike trains recorded by invasive BMIs and evaluated its performance offline with non-human primates performing virtual single finger movements (Benyamini et al., NER 2019). Here we extend this method to a more complex task involving simultaneous and independent movements of two finger-groups (index and middle-ring-small [MRS] fingers) and evaluate its performance both off and online.
Methods: We implanted one nonhuman primate with a Utah microelectrode array in the hand area of primary motor cortex. Following online brain-control of the virtual fingers using a Kalman filter (Nason et al., bioRxiv 2020), we trained step-wise linear discriminate analysis (SWLDA) classifiers to detect erroneous movements using thresholding events from 96 M1 electrodes in 50 ms bins. Short movement segments that were consistently toward or away from the target were labeled accordingly, while segments with inconsistent movement direction were not included in training. The effect of the number of bins in each segment was assessed by evaluating the true positive rate (TPR, i.e., the rate at which segments away from the target are correctly classified as such) and false positive rate (FPR, i.e., the rate at which segments toward the target are mistakenly classified as away from the target). Classification rates were improved by accounting for the decoded direction of the movement (extension or flexion), so two classifiers were trained for each finger-group resulting in 4 classifiers in total.
Receiver operating curves (ROCs), which quantify the tradeoff between TPR and FPR, were estimated for each classifier. We determined both the optimal performance, which minimizes total error rate, and the performance when, for practical operation, the FPR was restricted to 5%.
Results: Offline analysis revealed that best performance was achieved with 5 bins per segment (i.e., 250 ms windows). Optimal TPR and FPR were 82% and 34.3% for the MRS group and 68.4% and 46.5% for the index finger. Restricting FPR to 5% during validation resulted in a TPR

and FPR of 42% and 4.5% for the MRS group and 23% and 8% for the index finger. Real-time performance is under evaluation.
Conclusions: Erroneous movement segments can be distinguished from correct movements based on the neural activity in M1. This suggests that the neural activity encodes information related to error processing and can be used to improve invasive BMIs.

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Poster

P556. Neuroprosthetics: Control of Real and Artificial Arm, Hand, Other Grasping Devices II

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Topic: E.05. Brain-Machine Interface

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Kahn AWD011321

Title: A pulse-interval modulation communication scheme for low-power, wireless brain machine interfaces

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Abstract: Brain-machine interfaces (BMIs) have the potential to restore motor function but are currently limited by electrode count and long-term recording stability. These challenges may be solved through the use of free-floating “motes” which wirelessly transmit recorded neural signals. In particular, motes that are powered by and transmit data through infrared light can be scaled to sub-250 um dimensions for high-density recording, a similar density to existing arrays. However, a key challenge with implanted wireless devices is minimizing power usage to stay within safe tissue irradiation limits.
Here, we evaluate a novel pulse-interval modulation (PIM) communication scheme that reduces mote power usage to stay within limits. It has previously been shown that recording power can be reduced by sampling at only 2000 Hz and using the 300-1000 Hz spiking band power (SBP)

as a neural feature. With the PIM scheme, data packets contain each mote's ID and encode SBP by the rate at which packets are transmitted (Lim...Blaauw, VLSI 2021). Then, a receiver can resolve packet collisions through the use of temporal and spatial filters matched to each mote's ID. Individual mote power usage and signal fidelity can be adjusted by changing the average packet rate. Due to asynchronous transmission times, PIM-based motes can use clock speeds less than 10 kHz, unlike synchronized schemes that require clock speed (and power) to scale with the number of motes. Furthermore, PIM removes the need for a power-demanding analog-to-digital converter.

To test PIM's ability to efficiently communicate SBP, we simulated the communication scheme in a real-time BMI. Two Rhesus macaque monkeys, previously implanted with 96-channel Utah microelectrode arrays in primary motor cortex, were trained to control a virtual hand using the BMI. In this system, Kalman filter decoders predicted finger velocity from SBP or simulated PIM-SBP. At a rate of 100 packets/s, equivalent to 1.3 Kbps using a 12-bit mote ID, PIM-SBP decoders performed as well as standard SBP decoders (target acquisition times of 1.26 and 1.36 sec, respectively). This suggests that BMI performance can be maintained with significantly lower bit rates than transmitting the full 30 kHz signal (480 Kbps/channel) or SBP signal (32 Kbps/channel). Using this scheme, data from 1000 channels could be transmitted using 1.3 Mbps, and initial simulations of 1000 motes suggest that despite packet collisions, SBP data can be recovered through the use of matched filters on the receiver.

These results suggest that PIM-based communication can significantly reduce power usage of wireless motes to enable higher channel counts for high-performance BMIs.

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Poster

P556. Neuroprosthetics: Control of Real and Artificial Arm, Hand, Other Grasping Devices II

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Weill Institute for Neuroscience

Title: ECoG based BCI control of reaching and grasping with a robotic arm using stacked discrete commands

Authors: *S. SEKO[1], N. NATRAJ[1], R. ABIRI[1], Y. GRAHAM[1], N. HARDY[1], E. F. CHANG[2], K. GANGULY[1];
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Abstract: Brain Computer Interfaces (BCIs) have the potential to restore lost upper limb function by translating neural signals into the motion of an external device. Despite successful demonstrations, there remain challenges to real-world BCI control of complex tasks, including signal instability. Additionally, continuous, high-dimensional neural input is required to perform functional upper limb tasks which operate in a high dimensional action space. Recently, we demonstrated that stable "plug-and-play" control of a 2D cursor is possible by leveraging the stability of electrocorticography (ECoG) with long-term closed-loop decoder adaptation. Here, we aim to increase the control dimension of the action space by using multiple stable and stacked discrete signals with the goal of continuous, real-time control of a 6-dof robotic arm. A subject with severe spastic tetraparesis was implanted with a 128-channel chronic ECoG array over sensory and motor cortices. To initialize discrete control commands, the subject was instructed to imagine movements related to distinct body parts (right hand, head, left hand, feet, tongue, and lips). A multilayer perceptron was used to classify discrete actions from the extracted neural features (delta (0.5-4 Hz), beta (12-30 Hz), and high-gamma (70-150 Hz) power). The discrete actions were mapped to Cartesian directions to control the translation of a robotic arm end-effector. Continuous motion was achieved by imposing virtual mass-damper dynamics with the decoded discrete state acting as an input. This produces smooth motion with the ability to move in off-axis trajectories. To enable robotic grasping, the same set of imagined movements was mapped to an alternate set of robot actions that control the gripper opening and wrist orientation. An additional imagined movement (both hands) acts as a clicker, allowing the subject to switch between the translation and grasp modes and increasing the dimension of control. Using this framework, the subject was able to successfully control the robotic arm to reach to and grasp objects on a table. These results suggest that robust discrete commands decoded from ECoG signals can be used as the input to be a "universal joystick" for neuroprosthetic control which can be mapped to different action spaces, providing a framework for the development of long-term control of devices which operate in high-dimensional spaces.

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Poster

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Title: High-velocity prosthetic finger movements in real-time brain-machine interfaces using artificial neural networks

Authors: *M. S. WILLSEY[1], S. R. NASON[1], S. R. ENSEL[4], H. TEMMAR[1], M. J. MENDER[1], J. T. COSTELLO[1], P. G. PATIL[2], C. A. CHESTEK[3];
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Abstract: Brain-machine interfaces (BMIs) that control prostheses may help restore motor movements to patients with injuries causing motor paralysis. The algorithm converting recorded neural activity into motor movements for the prostheses, the decoder, is critically important to its speed and efficiency. Herein, we introduce a novel feed-forward neural network velocity decoder (NN) to control finger prostheses using neural activity recorded from intracortical Utah arrays. An intention based retraining step, similar to that used in the recalibrated feedback intention–trained (ReFIT) Kalman filter, was used to produce an improved decoder, the ReFIT NN (RN). Utah arrays were implanted in motor cortex of two male rhesus macaques, trained to perform a two-dimensional finger task. Using a low power proxy for spiking rate, spike-band power (SBP), a five-layer neural network decoder (NN) was trained based on the relationship between finger velocities and SBP. NN was then used to decode finger trials and further refined by the ReFIT re-training step. Over multiple days of testing in both animals, the NN and RN are compared using an adaption to Fitt's law bit rate, a measure of throughput, without online hyperparameter tuning. The NN and RN decoders are then compared to our previously published ReFIT Kalman filter for fingers (RK; Nason et al. 2020). In both animals, when comparing RN vs NN, the intention based retraining step substantially improved the throughput – 42% in Monkey N ($P < 10^{-5}$) and 20% in Monkey W ($P < 10^{-5}$). Comparing RN with RK also revealed performance gains of 62% in Monkey N ($P < 10^{-5}$). RN was found to operate with higher decoding velocities than RK, and these higher velocities are thought to account for the improved performance. An offline analysis accounting for differences in decoder gain also confirmed that the neural network algorithm was better able to model higher velocities than the Kalman filter. Using an architecture loosely inspired by biological motor pathways, this novel neural network decoder can be refined with an intention-based retraining step and substantially outperforms our previously published RK with more natural-appearing finger movements.

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Poster

P556. Neuroprosthetics: Control of Real and Artificial Arm, Hand, Other Grasping Devices II

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Topic: E.05. Brain-Machine Interface

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NIH Grant T32AR007505-33

Title: Improving reaching with functional electrical stimulation by incorporating stiffness modulation

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Abstract: Objective: Intracortical recordings have now been combined with functional electrical stimulation (FES) of arm/hand muscles to demonstrate restoration of upper-limb function after spinal cord injury. However, for each desired limb position decoded from the brain, there are multiple combinations of muscle stimulation levels that can produce that position. The objective of this simulation study is to use a virtual arm model to explore how modulating the amount of coactivation of antagonist muscles can impact reaching performance and energy usage during FES. Stiffening the limb by cocontracting antagonist muscles makes the limb more resistant to perturbation. Minimizing cocontraction saves energy and reduces fatigue. Approach: Prior demonstrations of reaching via FES used a fixed empirically-derived lookup table for each joint that defined the muscle stimulation levels that would position the limb at the desired joint angle decoded from the brain at each timestep. This study expands on that previous work by using simulations to: A) test the feasibility of controlling arm reaching using a suite of lookup tables with varying levels of cocontraction instead of a single fixed lookup table for each joint, B) optimize a simple function for automatically switching between these different cocontraction tables using only the desired kinematic information already being decoded from the brain, and C) compare energy savings and movement performance when using the optimized function to automatically modulate cocontraction during reaching versus using the best fixed level of cocontraction. Main Results: Our data suggests energy usage and/or movement performance can be significantly improved by dynamically modulating limb stiffness using our multi-table method and a simple function that determines cocontraction level based on decoded endpoint speed and its derivative. Specifically, reducing cocontraction during acceleration and increasing cocontraction during deceleration enabled one to reduce the baseline level of cocontraction and energy usage while still maintaining good movement performance. Significance: By demonstrating how modulating cocontraction can reduce energy usage while maintaining or even improving movement performance, this study makes brain-controlled FES a more viable option for restoration of reaching after paralysis.

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Poster

P556. Neuroprosthetics: Control of Real and Artificial Arm, Hand, Other Grasping Devices II

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Topic: E.05. Brain-Machine Interface

Support: USAMRAA (W81WXH1910839)

Title: Decoding Recordings of Extracellular Activity from Longitudinal Intrafasicular Electrodes to Control a Prosthetic Hand

Authors: *T. R. BENIGNI[1], J. J. ABBAS[2], R. JUNG[1];
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Abstract: Motorized prosthesis users typically control their prosthesis with signals derived from muscles in the residual limb. Neural recordings from longitudinal intrafascicular electrodes (LIFEs) have been used to control a single degree of freedom prosthesis using a Schmitt trigger and leaky integrator to provide some level of control. With the introduction of multi-degree of freedom prostheses with options for grasp patterns and improved capabilities for graded control, advanced algorithms will be required to simultaneously decode multiple graded motor intent signals in order to detect the class of the action (e.g. grasp type) and the degree of the action (e.g. grasp force). We have developed a Python-based algorithm suitable for real-time decoding of motor intent recorded from small groups of axons. To assess the quality of the decoding algorithm, we used our published software platform to simulate neural motor signals recorded with LIFE electrodes. The simulator utilizes motor intent signals to drive a mixed pool of simulated motoneurons with various spike shapes, recruitment characteristics, and firing frequencies. Each electrode records a weighted sum of a subset of simulated motoneuron activity patterns allowing the simulated electrodes to be influenced by multiple motor pools (Abdelhani et al. 2014). Three different motor intent signals commonly used to control a prosthesis were used to generate simulated extracellular recordings to assess the decoding algorithm's performance: a grip and hold (saturating line function), a grip, hold and drop (square pulse), and a low force open/close (cosine wave function). Recordings were generated with a sampling rate of 24 kHz and a good quality extracellular SNR of 5 dB. The decoding algorithm used small windows of the simulated neural recording (50 ms) and then filtered, rectified, and normalized each window, to extract signal features (mean absolute value) related to the motor intent. Control signals for prosthetic hands need to have an overall latency of less than 100 ms and have a high enough quality to transmit the user's intent. Results demonstrated that the decoder can process

each window at <2ms and match the underlying intent with an average normalized root mean square error of less than 16%. This indicates that the decoded signals could be suitable for the control of a prosthetic hand. We plan to investigate if this approach, when applied to multiple channels of LIFE recordings from antagonist motor pools, could be used to control a multi-degree of freedom prosthesis.

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Poster

P556. Neuroprosthetics: Control of Real and Artificial Arm, Hand, Other Grasping Devices II

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Topic: E.05. Brain-Machine Interface

Support: NIH NS102259

Title: Restoration of complex movements in paralyzed upper limbs

Authors: *B. A. HASSE, D. E. G. SHEETS, N. L. HOLLY, K. M. GOTHARD, A. J. FUGLEVAND;
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Abstract: Functional electrical stimulation (FES) artificially activates muscles to help restore function in paralyzed limbs. The use of FES to enable reaching and grasping in tetraplegics has been limited to a small set of preprogrammed movements due to the difficulty in identifying stimulus patterns needed to elicit more complex behaviors. To overcome this limitation, we used machine learning to predict patterns of activity across multiple muscles associated with complex upper limb movements. Those patterns were then used as FES templates to evoke desired arm movements in temporarily paralyzed (anesthetized) monkeys. Twenty-nine arm muscles in each of three male rhesus macaques were surgically implanted with recording/stimulating electrodes. Electromyographic (EMG) and kinematic data, recorded while the monkeys tracked complex trajectories, were used to train artificial neural networks (ANNs). Trained ANNs were then used to predict EMG signals associated with a new set of complex movements. Predicted patterns of muscle activity were transformed into stimulus pulse trains that were subsequently delivered to intramuscular electrodes to evoke arm movements in the monkeys while under general anesthesia. Predicted EMG signals were based on training data obtained from the same monkey (*'self'*) or from a different monkey (*'other'*). The accuracies of *self*-predicted EMG for muscles actively involved in moving the hand through space were quite good with R^2 values often > 0.8. As expected, *other*-predicted EMG signals for such muscles were not quite as good but still reasonable with $R^2 \sim 0.60$. When predicted (*self* and *other*) or *actual* EMG signals were used as

stimulation templates, the average R^2 values for evoked compared to desired kinematics were not significantly different from one another (all ~ 0.33). However, all of these were significantly greater than that associated with evoked movements in which the stimulus patterns were randomly shuffled among all muscles. These results indicate that the sources of EMG templates are not crucial, and that errors are primarily related to how such templates are transformed into muscle active states. Regardless, these findings demonstrate that machine learning can be used to identify patterns of muscle stimulation needed to evoke complex, large scale movements in paralyzed upper limbs that approximate desired trajectories. The errors, however, can be significant - inclusion of real-time feedback would seem to be one way to improve control over these otherwise open-loop movements.

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Poster

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Topic: E.05. Brain-Machine Interface

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NSF-1901236

Title: Mirror Training Improves Offline-Decode Performance for Multi-DOF Myoelectric Prosthetic Control

Authors: *T. N. TULLY, C. J. THOMSON, G. A. CLARK, J. A. GEORGE;
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Abstract: Transradial prostheses can provide intuitive control by training an algorithm to correlate electromyographic signals from the residual forearm muscles to intended movements of the amputated hand. One training paradigm, “mimicked training”, relies on amputees mimicking a virtual hand with their missing hand such that the corresponding muscle activations are correlated to the preprogrammed kinematics. A second training paradigm, “mirrored training”, relies on unilateral amputees mirroring their contralateral hand with their missing hand such that the muscle activations are correlated to the kinematics of the contralateral hand (determined via motion capture). Our prior work with intact participants demonstrated that mirrored training captures kinematics more closely related to the target (i.e., missing) hand. This abstract extends this work to assess the impact on prosthetic control. Seven participants mimicked a virtual bionic

hand with their dominant hand and contralateral hand simultaneously while we recorded the kinematics of both hands with a low-cost infrared camera. We compared the performance of a modified Kalman filter (MKF), shallow convolutional neural network (CNN) and a deep CNN for both training paradigms. We also compared the performance of these 3 algorithms when the training dataset was small (5 movements per digit) versus large (50 movements per digit). Performance was measured as the Root Mean Squared Error of algorithm predictions relative to the ground-truth kinematics of the dominant hand. The larger training dataset significantly improved the performance of both CNNs under both training paradigms (p's < 0.0042, individual t-tests with Dunn-Sidak correction for multiple comparisons), whereas there was the larger dataset did not improve the MKF under either training paradigm. For the large dataset, mirrored training significantly improved the shallow CNN and deep CNN (p's < 0.0042. For the small dataset, mirrored training significantly improved the shallow CNN ($p < 0.0042$) and trended towards improving the deep CNN ($p = 0.0278$). Further investigations will explore a larger demographic of intact subjects, examine real-time performance, and compare these training paradigms and algorithms with amputee participants on activities of daily living.

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Poster

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Topic: E.05. Brain-Machine Interface

Support: NIH Grant (1 F31 NS110364-01A1)

Title: Object property recognition during closed-loop control of a prosthetic hand with elicited tactile feedback through transcutaneous nerve stimulation

Authors: *L. VARGAS[1], H. HUANG[1], Y. ZHU[2], X. HU[3];
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Abstract: Although many prosthetic devices have evolved to replicate the motions of the human hand, these systems often lack tactile feedback to the users. This study aimed to evaluate if non-invasive somatotopic feedback elicited through transcutaneous nerve stimulation could help

participants to discern different object properties (shape and size), while a prosthetic hand was controlled using different myoelectric control strategies (position and velocity control). Using a 2x8 electrode array placed on the upper arm, fingertip tactile sensations were elicited through stimulation of the median and ulnar nerves. Evoked percepts were intensity-modulated to resemble the changes in fingertip forces from a prosthetic's index and middle fingers. Myoelectric signals of the finger flexor and extensor muscles were used to control the position or velocity of the prosthetic finger joint. Object shape recognition involved the stimulation of two distinct electrodes pairs each corresponding to discrete fingers. Object size was distinguished based on the time to contact and/or the level of muscle activity during position control. The results showed a significant improvement of the object recognition accuracy with evoked tactile feedback. Our results demonstrated the efficacy of our nerve stimulation approach and its robustness across control strategies. Overall, the outcomes promote our understanding on the role of sensory feedback during human-machine interactions, potentially improving user experience and confidence when using myoelectric prosthetic hands.

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Poster

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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: Influence of sensory feedback signals on motor cortex during hand movement using a soft robotic glove

Authors: *J. T. GUSMAN[1,2,4], D. S. DE LUCENA[5], C. E. VARGAS-IRWIN[3,2,4], A. KAPITONAVA[6], D. A. WAGNER[5], T. HOSMAN[1,2,4], C. WALSH[5], L. R. HOCHBERG[4,1,6,7,2]; [1]Sch. of Engin., [2]Carney Inst. for Brain Sci., [3]Dept. of Neurosci., Brown Univ., Providence, RI;

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Abstract: Intracortical brain-computer interfaces (iBCIs) use signals recorded directly from the brain to help individuals with paralysis to control assistive devices such as computer cursors or robotic arms. Robotic exoskeletons, which are typically made out of rigid materials combined with motors and gears, have limitations as iBCI effectors due to their relative complexity, bulkiness, and cost. Recently, advances in soft robotic technology have enabled the development of soft exoskeletal wearables that have shown promise as assistive and rehabilitative tools for individuals with motor impairment. In this study, we paired our iBCI neural recording and decoding system with a soft robotic glove (SRG) and explored the effects of proprioceptive feedback during SRG-activated hand movement on neural activity in the human motor cortex. Data were collected from participant T11, a 36-year-old, right-handed man with a spinal cord injury (C4 AIS-B). T11 had two 96-electrode arrays (BlackRock Microsystems) implanted in his left precentral gyrus (PCG) as part of the BrainGate Clinical Trial. Neural data were recorded while T11 was asked to attempt functional hand movements (power grip, pinch grip, open hand) while the quality of sensory feedback was varied. An SRG moved either his own hand or a mannequin hand into the corresponding grip postures. Moveable barriers were used to either obstruct or expose visual feedback of the moving hand. T11 was also asked to perform trials where he passively observed the moving hand without attempting the grips. Threshold-crossing rates were used to assess neural activity across 192 channels and construct neural decoders to assess discriminability between different hand movements. The recorded neural population responded to all volitional and feedback conditions, including conditions where the participant experienced visual feedback or proprioceptive feedback alone. Neural responses during attempted hand movement were more robust than during passive observation, and visual feedback evoked greater neural modulation than proprioceptive feedback. Neural decoders were able to reliably discriminate between grip types when calibrated on attempted movement data and could also decode passively-watched hand movements but with less accuracy. The type of feedback did not significantly affect decoding of grip type during attempted movement trials, however, suggesting that the introduction of proprioceptive feedback produced when operating an SRG would not negatively impact decoding in a PCG-based iBCI. Further work is needed to verify these results in individuals with different degrees of upper-limb sensorimotor impairment.

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Poster

P556. Neuroprosthetics: Control of Real and Artificial Arm, Hand, Other Grasping Devices II

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The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH, the Department of VA or the US Government. Caution: Investigational Device. Limited by Federal Law to Investigational Use.

Title: Interaction of effector and direction encoding in motor cortex of a person with tetraplegia during a bimanual task

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Abstract: Intracortical brain computer interfaces (iBCIs) have consistently enabled individuals with paralysis to control a computer cursor or a robotic arm and hand. Simultaneous decoding of bilateral movements would offer additional degrees of freedom to interact and communicate through iBCI assistive technologies, such as two-handed typing, tablet control using gestures of each hand, or twisting open a jar. Here, we report intracortical neural encoding in human motor cortex during bilateral movements when the effector (right or left thumb) and movement direction (upper, lower, left, or right targets) are cued separately.

At the time of this study, the participant, T11, was a 36 year-old male with C4 ASI-B spinal cord injury who had two 96-channel microelectrode arrays implanted in the left precentral gyrus. In an instructed delay task, T11 attempted to move a single cursor from the center to one of four cardinal targets with his left, right, or both thumbs. The upcoming target direction and effector instructions were presented sequentially. Center-out cursor movements were controlled by the computer and analyses were performed offline with two types of features derived from neural activity: binned spiking (threshold crossings at 3.5 RMS) and spike-power band LFPs (250-5000 Hz). Significance was assessed using the Kruskal-Wallis test (p<0.01).

For cue order direction then effector choice, we found that, on average, 50% of 384 features deviated from baseline values (i.e. were considered responsive) when direction cues were

presented without effector context and 29% of features then displayed significantly different values for different targets (i.e. were direction selective). Next, when the effector was presented, 52% of features were responsive and 8% selective between left and right movement trials. Interestingly, during unimanual ipsilateral left/right movement trials ~75% of the selective feature responses changed after the effector cue presentation suggestive of a body-midline representation. No equivalent change in response was found during contralateral or bimanual left/right movements nor for any effector during up/down movements. We additionally performed ensemble analyses to test for a communication subspace where movement and effector information is combined. Finally, we applied the same techniques to a similar partially cued grasp task. While this study was performed with a single participant with tetraplegia, these results suggest that the effector context is important for understanding and decoding bimanual movements for iBCIs.

Disclosures: T. Hosman: None. **C.E. Vargas-Irwin:** None. **A. Kapitonava:** None. **L.R. Hochberg:** Other; Neuralink, Paradromics, Synchron. **J.D. Simeral:** None.

Poster

P561. Posture and Gait: Afferent Control

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P561.01

Topic: E.06. Posture and Gait

Title: Internal Model with Interaction Gain and Feedback Contribution Successfully Interprets Weight Distribution According to the Asymmetry in Plantar Pressure

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Abstract: The variety and steadily increasing quantity of cases where patients have motor impairments is prompting the development of new rehabilitative techniques. Sensory intervention is promising as motion dependent sensory feedback plays a critical role in reorganizing the nervous system and recovering motor function. To maximize the efficacy of existing rehabilitative techniques and promote the development of new approaches, a full understanding of how the human sensorimotor system responds to the given sensory intervention is essential. The leading method for understanding the sensory intervention is through the analysis based on an internal model, which analytically describes the operation of the sensorimotor system based on known neural pathways. However, currently available internal models do not yet provide sufficient details to quantitively characterize the sensorimotor loop operation, and therefore are not actively used by clinicians. The objective of this experiment was to develop a new internal model which can quantitatively describe the change in motor output in response to the sensory intervention. To accomplish this goal, we added “interaction gain” and

"feedback contribution" to the internal model and identified those values based on the relationship between the change in plantar pressure (sensory intervention) and weight distribution (motor output). Those values are defined respectively as the level of interaction the sensorimotor system has with the associated stimulus and as the amount of feedback control that affects the associated motor output. A balancing task was chosen to demonstrate the new internal model. Subjects stood on rubber mats of varying surface areas on top of a pressure pad, to change the plantar pressure. Data was collected for both blindfolded and unblindfolded cases, and both cases where the subjects were instructed to balance normally and where subjects were instructed to distribute weight to equalize plantar pressure. From these results we determined that (1) tactile feedback contribution was significant for the cases where the subject was instructed to match the perceived plantar pressure; (2) tactile feedback contribution was larger for trials where visual feedback was restricted; (3) visual feedback contribution increased when subjects focused on matching tactile pressure. Based on the results, we conclude that the new internal model with interaction gain and feedback contribution will discover how the motor output changes based on sensory intervention.

Disclosures: D. Dollahon: A. Employment/Salary (full or part-time):; Texas A&M University.
H. Park: A. Employment/Salary (full or part-time):; Texas A&M University.

Poster

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Program #/Poster #: P561.02

Topic: E.06. Posture and Gait

Support: K12 HD055931 to HEK

Title: Methods to test lower limb position sense using a split belt treadmill

Authors: *J. WOOD, H. E. KIM, S. M. MORTON;
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Abstract: Human locomotion is highly adaptable, allowing us to move across a range of different environments and surfaces. To safely walk under these varied conditions, it is necessary to have accurate estimates of lower limb location which often rely less on visual feedback, making our position sense estimates all the more important. However, it is unclear how accurate lower limb position sense estimates are and whether perceptions shift during gait adaptation. Locomotor adaptation is frequently studied using the split-belt treadmill, in which the two treadmill belts, and therefore the two legs, are driven at different speeds, such that participants are forced to walk with a significant degree of interlimb asymmetry. The most consistent sensory change observed after adapting in this paradigm is the sense of limb speed (Jensen et al., 1998,

Leech et al., 2018). Yet, other, perhaps equally important percepts such as limb position sense, i.e., one foot's position relative to the other, have received less attention. Therefore, the objective of the current study was to assess the accuracy of relative foot positions measured in a locomotor-relevant task to provide a basis for future assessments of proprioceptive shifts caused by locomotor adaptation. We modified the approach used by Vasquez et al. (2015). We employed a two-alternative forced choice task using the method of constant stimuli with 9 target positions (0, ±10, ±20, ±30, ±40 mm) representing the difference between the two legs along the sagittal plane. Individuals stood on a treadmill with vision of their legs occluded. For each trial, the treadmill slowly moved the test limb to a random start position drawn from a normal distribution centered on zero (to prevent the limb always moving in the same direction) then to the target location. Each target was visited 15 times. Participants were asked if they felt the left or the right limb was more forward. We calculated the probability of the participant responding 'left' at each target location and fit a psychometric function to each individual's data. We used the mean and standard deviation of the psychometric fits as our measures of bias and precision of limb position sense, respectively. We found that, on average, participants in our sample perceived their test limb to be slightly behind their reference limb, and their position was accurate to < 1 cm. We conclude that this method can be used to determine perceptual shifts induced by split-belt treadmill adaptation.

Disclosures: J. Wood: None. **H.E. Kim:** None. **S.M. Morton:** None.

Poster

P561. Posture and Gait: Afferent Control

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Program #/Poster #: P561.03

Topic: E.06. Posture and Gait

Support: Unidel Distinguished Scholars Fellowship

Title: Responses to visual perturbations during walking in children with and without cerebral palsy.

Authors: *A. SANSARE[1], M. ARCODIA[2], S. C. K. LEE[1], J. J. JEKA[3], H. REIMANN[2];
[1]Dept of Physical Therapy, [2]Kinesiology and Applied Physiol., [3]Kinesiology & Applied Physiol., Univ. of Delaware, Newark, DE

Abstract: Children with cerebral palsy (CP) tend to rely on vision more than other sensory modes to control their balance in standing and during single plane lower extremity movement. However, it is not known whether this also applies to walking, an activity where the threat to balance is generally higher. The aim of this pilot study is to assess how children with CP use visual information for sensorimotor feedback during walking, compared to children with typical

development (TD). We perturb balance during treadmill walking by visual fall stimuli in the frontal plane using a virtual reality projection. Our hypothesis, based on previous studies on older adults, is that children with CP will demonstrate a magnified and delayed response to visual perturbations. Additionally, unlike neurotypical adults who show stance foot ankle roll as an immediate response to visual perturbations, followed by a step placement response, we hypothesize that children with CP would show a lack of ankle roll but a larger step placement response to the visual perturbations. Twelve children (6 with CP, age 18 ± 2.9, 2 female; 6 age- and sex-matched controls with TD, age 18 ± 3.1) participated in the study. They walked on a self-paced treadmill inside a virtual reality environment that induced a visual fall stimulus in the frontal plane every 10-12 steps. Each trial lasted two minutes and was repeated ten times, with intermittent rest breaks. Participants in both CP and TD group reacted to the perturbation by moving their center of mass away from the fall stimulus. Participants with CP showed a delayed and magnified shift in the center of mass trajectory compared to their age-matched TD peers. While the participants with TD inverted their ankle immediately following a visual fall stimulus, the participants with CP did not show this response, as indicated by no change in their subtalar joint angles and peroneal muscle electromyography recording. Instead, they employed a magnified step placement strategy, showing a higher peak step placement change compared to the TD group. Overall, a magnified and delayed response to visual perturbations was seen in participants with CP compared to their age and sex matched peers. These results suggest that children with CP show a greater reliance on visual feedback over other sensory modalities, potentially to compensate for impaired proprioceptive abilities seen in CP. While most assessments as well as treatments for balance rehabilitation in CP address motor deficits, our findings highlight the importance of considering sensory regulation of walking balance in rehabilitation protocols.

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Poster

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Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P561.04

Topic: E.06. Posture and Gait

Title: Purkinje cell modulations of vestibular neurons and its role for balance

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Abstract: The cerebellum is important to adjust and maintain balance. Consequently, lesions in the vestibular cerebellum lead to postural instabilities. While it is known that Purkinje cells (PC)

of the cerebellum directly target neurons in the vestibular nuclei, it is unclear how these vestibular neurons are modulated by PCs in the context of balance and posture. Zebrafish are an exceptional model system to address this question. Our lab has done much work to characterize the vestibular circuitry of larval zebrafish and develop tools to measure vestibular function and postural control. Here we report a set of chemogenetic manipulations to directly activate or lesion PCs and the resulting effect on postural control, as well as calcium imaging to further dissect this circuit. After PC lesion, fish adopt asymmetric postures in the pitch axis. Specifically, we observed PC lesioned fish spent more time at eccentric nose-up postures than control siblings. Further, we observed asymmetric implementation of postural adjustments. We used a generative model of swimming to show that changes to these corrective postural adjustments can account for the nose-up postures we observe. To investigate PC modulations, we are using a vestibular pitch stimulus combined with calcium imaging in vestibular nucleus neurons. We give a nose-up stimulus or a nose-down stimulus. The majority of cells have a preferred stimulus direction, either nose-up or nose-down. After lesion of PCs, we observe systematic changes in the calcium responses of putatively PC-targeted neurons in the vestibular nucleus. Nose-down responses increased in magnitude after lesion, indicating direct Purkinje cell input, whereas nose-up responses decreased in magnitude, suggesting indirect input via contralateral, inhibitory neurons. Together, we find an asymmetric effect of PC lesions on behavior as well as on downstream targets, likely explained by an asymmetry in targeting of nose-up versus nose-down preferring cells.

Disclosures: F. Auer: None. **K. Nardone:** None. **D. Schoppik:** None.

Poster

P561. Posture and Gait: Afferent Control

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Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P561.05

Topic: E.06. Posture and Gait

Title: The Role of Cognitive Impairment on Dual-Task Gait Performance

Authors: *D. GHIDELLA, T. PHAM, Z. TUTTLE, V. KRISHNAN, Y.-H. CHO;
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Abstract: More than 33% of older adults age 65 and older fall each year, with fall risk increasing each year (Gillespie et al., 2009). Falls often occur when dual-task (DT) walking (walking while performing a cognitive task) where DT gait cost (difference between walking with and without performing a cognitive task) was reported to be positively associated with future falls (Muir-Hunter & Wittwer, 2016). Previous studies have used postural stability and walking speed as measures of DT gait cost (Yogev-Seligman et al., 2008). However, it is uncertain whether other gait parameters may be linked with future fall risk (Muir-Hunter &

Witterwer, 2016). Our study will bridge the gap within the literature by investigating the roles of various levels of cognition on DT cost of multiple gait parameters. We hypothesized that cognitively healthy older adults will have better gait performance than the mildly and severely impaired group. Sixty-four older adults (M_{age} = 76.2 ± 8.23) participated in the study. Global cognition was assessed using Montreal Cognitive Assessment (MoCA). Older adults were categorized as cognitively healthy (N = 27), mildly impaired (N = 25), severely impaired (N = 12) if their MoCA score was ≥ 26, between 22 - 25, and ≤ 21, respectively. Gait parameters included velocity step length, stride length, cycle, and cycle time coefficient of variation (CV) and were measured using Zeno walkway system. DT gait cost was computed from single-task (ST; walking alone) and dual-task (walking while performing Stroop) conditions with this formula: [((DT - ST) / ST) * 100]. One-
way MANOVA was conducted to examine the role of level of cognitive impairment on the combined DT cost of the gait parameters. Differences among the groups on the DT gait cost were statistically significant, Wilks' Λ = .71, $F(10, 114) = 2.11$, p=.029. Follow up univariate analysis showed that the cognitively healthy older adults had less difficulty in walking during DT than the severely impaired older adults on velocity [F(2, 1376.28) = 6.312, p =.003)], step length [F(2, 354.48) = 3.318, p=0.43)], stride length [F(2, 363.47) = 3.369, p=.041)], cycle time F(2, 1074.38) = 3.1456, p = .0380], and cycle time CV [F(2, 153208.94) = 3.492, p = .037)]. The results indicate that older adults with severe cognitive impairment exhibited greater deficits in their gait than their cognitively healthy peers when dual-tasking. To the best of our knowledge, this study is one of the few to examine the association between different levels of cognition with various gait parameters among older adults. These findings can be extended by implementing different fall prevention programs according to older adults' level of cognitive impairment.

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Poster

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Title: Healthy aging modifies head kinematics during continuous gaze stabilization exercises

Authors: *W. H. SOUZA[1], O. ZOBEIRI[1], J. MILLAR[2], M. C. SCHUBERT[3], K. E. CULLEN[1]; [1]Dept. of Biomed. Engin., [2]Dept. of Physical Med. and Rehabil., [3]Dept. of Otolaryngology, The Johns Hopkins Univ., Baltimore, MD

Abstract: The vestibular system is essential to maintain gaze, gait and postural control. Gaze stability exercises are prescribed for patients with vestibular hypofunction and often for patients with subjective reports of dizziness, yet normal vestibular function. Recent evidence shows that head kinematics during continuous gaze stabilization exercises can differentiate individuals with vestibular hypofunction from healthy controls, suggesting these exercises may provide a reliable proxy of functional vestibular performance. It is unknown, however, the extent to which such difference in kinematic behavior is attributed to aging. Furthermore, given the high prevalence of dizziness and falls in healthy elderly, we sought to examine differences in head kinematics during yaw (horizontal) and pitch (vertical) head motion in young vs. older adults. Herein, we assess the effects of aging on head kinematics during continuous gaze stabilization exercises in adults with no vestibular impairment as per video head impulse testing. Twenty healthy participants (55% female) composed two groups: young (n = 10, 24 ± 2.7 years) and older (n = 10, 66 ± 5.4 years). Each performed six 30-second gaze stabilization exercises with continuous head motion in pitch (3) and yaw (3) axes while standing. Participants were asked to constantly look at a target – a 1-inch letter "x" placed at eye level on the wall (1m and 2m), or hand-held at arm's length. Motion signals were recorded with a 6-dimensional motion sensor (3-axis linear acceleration and 3-axis gyroscope) placed on the back of the participants' heads. We examined head range of motion and cycle duration. Range of motion was significantly reduced ($p < 0.05$ – 0.01) in older vs. younger participants in pitch axis only (1m-wall pitch, 1m-wall yaw and hand-held pitch and yaw exercises). Cycle duration was equivalent between groups. Our results showed that, while head rotation cycles are similar in time between groups, older adults deploy smaller amplitude motion to maintain gaze stability during pitch rotations – an adaptation implemented over gaze fixation on near, not far targets. This behavior suggests that healthy aging leads to slower head velocities as a strategy to ensure effective proximal gaze stability in pitch head rotation, even when vestibular function is effective. Moreover, head kinematic measurements during continuous gaze stabilization exercises reveal adaptations relative to older age, which should be accounted for by clinicians prescribing and interpreting this type of exercise.

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Poster

P561. Posture and Gait: Afferent Control

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Program #/Poster #: P561.07

Topic: E.06. Posture and Gait

Support: NIH Grant NS081118

Title: Neural pathways underlying thalamic deep brain stimulation-induced gait ataxia in Essential Tremor

Authors: M. BLUMENFELD[1], A. BRINDA[1], D. MARTICORENA[1], R. BUTLER[1], C. SPENCER[1], J. D. KRIEG[1], L. K. WILMERDING[1], T. PALNITKAR[2], R. PATRIAT[2], N. HAREL[2], L. SCHROCK[3], S. E. COOPER[3], J. Y. MATSUMOTO[3], *M. D. JOHNSON[1]; [1]Biomed. Engin., [2]CMRR, [3]Neurol., Univ. of Minnesota, Minneapolis, MN

Abstract: Background: Deep brain stimulation (DBS) in the region of the ventral intermediate nucleus (Vim) of the thalamus can be highly effective for treating tremor in individuals with Essential Tremor (ET). However, a side effect of DBS that can limit the ability to achieve full tremor control is gait ataxia.
Objective: In this study, high-field (7T) MRI-based patient-specific computational models of DBS were used to assess the relationship between induction of gait ataxia and activation of 12 different neural pathways in and around the motor thalamus.
Methods: Two ET patients (one with bilateral and one with unilateral Vim-DBS implants) performed trials of tandem gait during DBS across multiple electrode configurations and stimulation amplitudes. Trials were clinically evaluated as “ataxic” or “non-ataxic” based on the Brief Ataxic Rating Scale (BARS). For each implanted DBS lead, modeled tractographic pathways included the motor cortex (MC) to external Vim (VimE), MC to internal Vim (VimI), medial lemniscus (ML), MC to anterior ventral lateral thalamus (VLa), somatosensory cortex (SSC) to ventral posterior thalamus (VPL), superior cerebellar peduncle (SCP) to the reticular nucleus of thalamus, SCP to VimI, SCP to VimE, lenticular fasciculus (LF), thalamic fasciculus (TF), internal capsule, and zona incerta. These biophysical axon models were coupled with patient-specific finite element tissue models to estimate the percent activation of each of the twelve neural pathways for stimulation settings that were either ataxic or non-ataxic.
Results: While some differences in pathway activation between ataxic and non-ataxic gait were observed (increased ML and SSC-VPL activation and decreased LF and TF activation for ataxic settings), these differences were not statistically significant ($p>0.05$).
Significance: This preliminary data suggests that activation of a single neural pathway may not be solely responsible for production of gait ataxia as a side effect of Vim-DBS. Development of an objective measure of gait ataxia, such as using markerless pose estimation, may help further understand the relationship between pathway activation and gait ataxia.

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Poster

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Topic: E.06. Posture and Gait

Support: Brian's Hope
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Orphan disease Center

Title: Clustering and Prediction of Disease Trajectory in Adrenomyeloneuropathy

Authors: *B. R. TURK[1], Y. FAN[2], A. SMITH FINE[1], J. KELLER[1], M. UNBERATH[2], A. FATEMI[1];
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Abstract: BACKGROUND
Adrenomyeloneuropathy (AMN) is the slow progressive phenotype of X-linked Adrenoleukodystrophy (ALD). Dying-back axonopathy of the spinal cord and peripheral neuropathy underlie the neurological impairment in AMN, commonly presenting with sensory-motor deficits, ataxia, and pain. Rate of disease progression is variable in both men and heterozygous females with AMN.
AIM
This study aimed to A) explore the structure of AMN disease trajectories using unsupervised clustering methods, in order to determine whether labels for progression could be generated i.e. progressor vs non-progressor, and B) train a neural network to predict those individual patient labels.
METHOD
Longitudinal quantitative sensorimotor, gait measurement and clinical neurological data were gathered from 61 male and 87 female AMN from a clinical trial at the Kennedy Krieger Institute in 2007-2012 [NCT00545597]. An unsupervised pipeline was designed: I) Multi-cluster feature selection (MCFS) algorithm was applied to mean delta 1- and 2-year data-sets. II) binary K-means clustering was performed. III) Logistic regression for each variable, and variable domain (sensory, motor, mixed) was performed. Cluster difference between means were tested. The unsupervised pipeline was used to generate a binary pseudo-ground truth of AMN progression, which was then used to train a supervised Bayesian neural network (BNN) to predict disease progression. Leave-out-out cross-validation was used. In every fold, the unsupervised cluster was re-run, and then used to optimize BNN network parameters using cross-entropy between the network's class prediction and the unsupervised cluster assignment.
RESULTS
Generated clusters showed significant differences in gait measurements: trajectory, velocity, stride Length, cadence as well as hip strength, knee and ankle spasticity. There was no significantly different distribution of men and women, or between motor or sensory domain for

binary clustering. Interestingly, there was no correlation between EDSS (dropped from clustering) and trajectory clusters for 1 and 2 years. Cluster 1 with higher (more severe) mean trajectories was labelled ‘fast progressor/progressor’, and cluster 2 ‘slow progressor/non-progressor’. The BNN demonstrated an area under the ROC curve (AUC) of 0.904 for 1 year disease trajectory, and an AUC of 0.716 for 2-year disease trajectory.

CONCLUSION

We demonstrate that unsupervised clustering of AMN disease trajectory can be used to generate labels for progression, and that using those labels, a supervised neural network is able to confidently predict 1- and 2-year AMN disease progression.

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Poster

P735. Alcohol: Cognitive and Behavioral Effects

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH 1UH2-MH109168-01
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NIH 2T32AA014127

Title: Prenatal alcohol exposure alters power in translational neurophysiological signal during rodent touchscreen tasks

Authors: *S. OLGUIN[1], V. LICHERI[1], J. F. CAVANAGH[2], J. L. BRIGMAN[1,3];
[2]Dept. of Psychology, [1]Univ. of New Mexico, Albuquerque, NM; [3]New Mexico Alcohol Res. Ctr., Albuquerque, NM

Abstract: Although it is well established that alcohol consumption during pregnancy leads to long lasting difficulties in offspring, Fetal Alcohol Spectrum Disorders (FASD) remain the most common neurodevelopmental syndrome. Poor reporting of alcohol consumption throughout gestation and underdiagnoses of FASD requires novel diagnostic tools and improved behavioral measures between clinical and preclinical models to increase translation between species. The National Institutes of Mental Health (NIMH) has promoted the Research Domain Criteria (RDoC) initiative, which emphasizes the quantification of behavior more likely to reflect a specific neurophysiological circuit. Two RDoC domains that are negatively impacted in individuals with FASD are cognitive control utilizing 5-Choice Continuous Performance Task (5C-CPT) and reward learning via probabilistic learning task (PLT). Additionally, we tested physical effort using progressive ratio breakpoint (PRBP). These tasks can be integrated with

electroencephalography (EEG) recordings in humans and dura-resting EEG-like recordings in rodents. Here, we incorporated touchscreen analogs of these tasks with EEG-like recordings in a rodent prenatal alcohol exposure (PAE) model. Mice were obtained from the NMARC where PAE is established via a drinking-in-the-dark model (10% EtOH with 0.066% saccharine for 4hrs/day throughout gestation; BAC: ~90mg/dL; controls=0.066% sweetened water, "SAC"). Beginning at 8-10 weeks, mice trained to touch white square target stimuli of the 5C-CPT. Once responding rapidly and accurately mice underwent stereotactic surgery and fitted with dura-resting skull screws targeting medial prefrontal, parietal, and motor cortices, with a ground over the cerebellum. After recovery and reminder to criterion, non-target trials (5 stimuli presented; withholding of response rewarded) were added at a 2:1 ratio for 5 days, then recorded at a 5:1 ratio for 12 days. Animals were then recorded for 1 day on the PRBP, wherein animals had to touch a single stimuli for progressively more responses to receive reward. Lastly, animals were recorded for 5 days on PLT wherein the probability of reward differed for the target and non-target image. We found that PAE impaired the ability to withhold responding to non-targets during 5C-CPT and altered breakpoint on the PRBP. Importantly, when PAE animals displayed deficits in behavior, this tracked with alterations in power in relevant *a priori* regions of interest in the EEG-like signal. These results utilizing dura-resting recording suggests that alterations in cortical activity in readily available EEG systems can be used as a potential biomarker of PAE.

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Poster

P735. Alcohol: Cognitive and Behavioral Effects

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Program #/Poster #: P735.02

Topic: G.09. Drugs of Abuse and Addiction

Title: Focal repetitive electrical stimulation as an therapeutic intervention in a model of intermittent access to alcohol consumption in wistar rats

Authors: *A. LOPEZ-CASTRO, D. ANGELES-VALDEZ, J. ROJAS-PILONI, E. GARZA-VILLARREAL;
Univ. Nacional Autonoma De Mexico, Queretaro, Mexico

Abstract: About 3 millions of people died worldwide in 2018 due to Alcohol Use Disorder (AUD) which is defined as: excessive alcohol use that affects physical and mental health. Neuromodulation interventions, such as Deep Brain Stimulation (DBS) and repeated transcranial magnetic stimulation (rTMS), are proposed as possible new complementary therapies to treat AUD. However, information about mechanism of action, optimal brain targets and types of stimulation protocols remain insufficient. This study aimed to explore the effect of high-frequency electrical stimulation on the prelimbic cortex in subjects who developed chronic

voluntary alcohol consumption. Method: The two-bottle choice intermittent access AUD preclinical model (IA2BC) at 20% concentration is used in young adult wistar rats (P45) for 20 sessions and we will divide them based on the subjects of the groups, in high consumers and low consumers by means of the hierarchical cluster analysis. Subsequently, a high frequency stimulation protocol (20 Hz) is replicated for 10 consecutive days at 100 pulses (duration = 0.2μs intensity = 400μA) in eight randomized subjects, who were separated into: 1) an active stimulation group and 2) a group of simulated stimulation (sham). Results: Our results indicate that two subjects who received active stimulation and one with sham stimulation reduced their alcohol. consumption. Discussion: The two subject who reduced consumption where heavy alcohol users, the result of active stimulation could correspond to that of Henricks., et al (2019) who found that high-frequency electrical stimulation only modified high consumptions. And for the rat with sham stimulation that decreased consumption, it could be due to local morphological changes in the implantation area.

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Poster

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Program #/Poster #: P735.03

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant UO1 AA028710

Title: Frontocortical dependent behavioral deficits in rats exposed to binge levels of ethanol in adolescence are rescued following voluntary wheel running exercise

Authors: *M. J. FECIK, P. T. NUNES, L. M. SAVAGE, Ph.D.;
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Abstract: Exposure to binge levels of ethanol in adolescence alters the typical trajectory of this development and contributes to a number of neural and behavioral deficits that persist into adulthood, including damage to the basal forebrain cholinergic system, which is involved in modulating attention, arousal, and learning. The nucleus basalis magnocellularis (NbM) sends cholinergic projections to the entirety of the cortical mantle, including the medial prefrontal cortex (mPFC) and orbitofrontal cortex (OFC). One of the interventions thought to reverse some of the impairments seen in rats exposed to adolescent intermittent ethanol (AIE) is voluntary wheel running exercise (VEx). The current study looked to see if exposure to binge levels of ethanol during adolescence led to selective frontocortical behavioral deficits and whether or not these deficits could be rescued by VEx. 31 Long Evans rats of both sexes were assigned to either

an AIE group which received intragastric gavages of 20% ethanol dosed at 5mg/kg on a two day on-two day off schedule from PD 25-55, or a control group that received gavages of water on the same schedule. Rats from both the AIE and control group were further divided into a VEx group that was given free access to a running wheel attached to their cage from PD 70-100, or a stationary control group that stayed in their regular home cages during this time. Following their respective treatments, rats were tested on spontaneous alternation behavior using a plus shaped maze as well as attention set shifting behavior and a delayed non-matching to position task in an operant conditioning chamber. Our results show that AIE rats were impaired on reversal learning, an OFC dependent measure of behavioral flexibility, and that rats exposed to VEx performed significantly better than stationary controls on this task. We did not detect an AIE or VEx difference on either spontaneous alternation or delayed non-matching to position. These results form the basis for an ongoing project that will investigate these deficits with a larger sample size, as well as ACh efflux into the OFC and mPFC as measured by in vivo microdialysis and acetylcholinesterase fiber density in these regions.

Disclosures: M.J. Fecik: None. **P.T. Nunes:** None. **L.M. Savage:** None.

Poster

P735. Alcohol: Cognitive and Behavioral Effects

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P735.04

Topic: G.09. Drugs of Abuse and Addiction

Title: Oxytocin Attenuates Stress-Induced Reinstatement of Alcohol-Seeking Behavior in Rats

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Abstract: The National Institute of Health estimates that nearly 15 million people in the United States alone suffer from Alcohol Use Disorder (AUD). Stress responsivity often plays a major role in such alcohol-dependent behavior, and can trigger a relapse in addiction and usage. Therefore, it is important to investigate potential pharmacological interventions that may alleviate stress response during the addiction cycle. Previous studies have demonstrated that the neuropeptide oxytocin produces anxiolytic effects in mammals. The purpose of the present study was to investigate the effect of oxytocin on stress-induced alcohol relapse, and identify a neural structure mediating this effect, through the use of an ethanol self-administration and yohimbine-induced reinstatement paradigm. In Experiment 1 adult male Sprague-Dawley rats were first trained to self-administer ethanol through an FR1, and then FR3 schedule of reinforcement. An extinction period was then enacted in which a lever press no longer resulted in ethanol delivery. Stress-induced reinstatement of ethanol-seeking behavior was then induced by the anxiogenic drug yohimbine. Rats received either concurrent systemic administration of oxytocin (1 mg/kg;

i.p.) and yohimbine (2 mg/kg; i.p.) or yohimbine alone. While yohimbine administration resulted in reinstatement of ethanol-seeking behavior, concurrent administration of yohimbine and oxytocin attenuated this effect, suggesting that oxytocin may disrupt stress-induced ethanol-seeking behavior. The central amygdala (CeA) is a structure driving emotional responses and robustly expresses oxytocin receptors. In Experiment 2, animals received concurrent intra-CeA infusion of oxytocin (0.5µg/0.5 µL; intra-CeA) and systemic injection of yohimbine (2 mg/kg; i.p.) in an identical behavioral paradigm as Experiment 1. Intra-CeA oxytocin similarly attenuated yohimbine-induced reinstatement of ethanol-seeking behavior. These results demonstrate that oxytocin has the potential to attenuate stress-induced relapse into ethanol-seeking behavior, and that this mechanism is occurring specifically within the central amygdala.

Disclosures: H.S. Ballas: None. **S. Wilfur:** None. **K. Leong:** None.

Poster

P735. Alcohol: Cognitive and Behavioral Effects

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Program #/Poster #: P735.05

Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA Division of Intramural Clinical and Biological Research Z1A AA 000488
NIAAA Division of Intramural Clinical and Biological Research Z1A AA 000130

Title: Profiles of impulsivity and compulsive drinking and their associations with negative emotionality, alcohol consumption, and alcohol-related problems.

Authors: *T. GUNAWAN[1], U. ZIAUSYTE[2], J. W. LUK[2], J. K. MORRIS[1], M. L. SCHWANDT[2], V. A. RAMCHANDANI[1], N. DIAZGRANADOS[2], D. GOLDMAN[3]; [1]Lab. on Human Psychopharmacology, [2]Office of the Clin. Director, NIH/NIAAA, Bethesda, MD; [3]Lab. on Neurogenetics, NIH/NIAAA, Rockville, MD

Abstract: RATIONALE: Alcohol use disorder (AUD) is conceptualized as a transition from reward-driven, impulsive drinking to habit-driven, compulsive drinking. This leads to the possibility that distinct phenotypic clusters, or profiles, can be identified based on levels of trait impulsivity and compulsive drinking. The present study uses latent profile analysis (LPA) to identify such distinct phenotypic profiles and examine their relationships with negative emotionality, alcohol consumption, and alcohol-related problems. **METHODS:** Participants (*N* = 440; 40.5% female, 45.7% White) enrolled in the NIAAA natural history protocol approved by the IRB were diagnosed via SCID interview. 53.9% of the sample had a current AUD diagnosis and 40.0% were seeking treatment. Subscales of trait impulsivity (UPPS-P Impulsivity Scale), impaired control over drinking (Impaired Control Scale), and compulsive drinking (Obsessive-Compulsive Drinking Scale) were used as indicators in the LPA to identify distinct classes,

which were then used to examine associations with negative emotionality (anxiety, stress, depression), alcohol consumption (Timeline Followback), and alcohol-related problems (Alcohol Use Disorders Identification Test; AUDIT). Sex, age, race, income, education, treatment status, family history, and age of first drink were included as covariates. **RESULTS:** A four-profile solution provided the best fit to the data. Drinkers in profile 1 (n = 171; 39.0%) exhibited lowest impulsivity and compulsive drinking scores, profile 2 (n = 69; 15.6%) had moderate impulsivity but lower compulsive drinking scores, profile 3 (122; 27.8%) showed moderate impulsivity and compulsive drinking scores, and profile 4 (n = 78; 17.6%) displayed highest impulsivity and compulsive drinking scores. Negative emotionality scores were lowest among profile 1 and highest among profile 4. Profiles 2 and 3 exhibited similar levels of negative emotionality. Drinking days, heavy drinking days, and average drinks per day significantly increased across profiles, with profile 1 exhibiting the lowest alcohol consumption, and profile 4 exhibiting the highest. AUDIT scores followed the same pattern of effects. **CONCLUSIONS:** Our findings indicate 4 distinct profiles of drinkers based on their levels of trait impulsivity and compulsive drinking. These results are consistent with the conceptualization of AUD as a transition from reward-driven drinking to habit-driven drinking. These profiles may capture individuals at distinct stages of this transition. Future research is needed to examine the trajectory of these individuals across these profiles.

Disclosures: T. Gunawan: None. **U. Ziausyte:** None. **J.W. Luk:** None. **J.K. Morris:** None. **M.L. Schwandt:** None. **V.A. Ramchandani:** None. **N. Diazgranados:** None. **D. Goldman:** None.

Poster

P735. Alcohol: Cognitive and Behavioral Effects

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Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P735.06

Topic: G.09. Drugs of Abuse and Addiction

Title: The relationship of adolescent alcohol exposure with extinction learning and prefrontal parvalbumbin expressing neurons

Authors: *A. LENSING, R. BOERGER, S. GILBERT, H. LIN, C. PICKENS;
Kansas State Univ., Manhattan, KS

Abstract: There is an established link between adolescent alcohol consumption and various long-term behavioral changes. Our lab has previously found an association between adolescent alcohol consumption and operant extinction performance in adulthood, as rats that consumed more alcohol during adolescence showed faster extinction rates during reinforcer devaluation tests (Fisher et al., 2017). Since these studies were not specifically designed to test operant extinction learning, we conducted experiments to replicate this effect and determine a potential

underlying neural mechanism. Adolescent male and female rats received chronic intermittent access to alcohol (n = 23) or water-only (n = 19) access for 6 weeks (from postnatal day 26-66). Ten days after completion of the CIA paradigm, rats began behavioral training. The rats received 2 once-daily sessions of cued instrumental training, in which a lever-light compound was presented during 40-sec trial and lever-presses could earn 2 food pellets/trial on an intermittent reinforcement schedule. Then, rats underwent extinction training in which no food was earned. At the end of the study, parvalbumin-positive (PV+) neurons were assessed in medial prefrontal cortex subregions in both sexes. We found that rats with prior alcohol access exhibited faster instrumental extinction than rats only given water. There were no differences in PV+ expression between the groups given alcohol or water-only in the anterior cingulate cortex (ACC), prelimbic cortex (PL), or infralimbic cortex (IL). However, there was a significant interaction of sex and the level of alcohol consumption in the PL and ACC PV+ levels in rats with prior alcohol access. Within the alcohol exposed group, males had a positive correlation between PL PV+ neurons and the level of alcohol consumption while females had a weak negative correlation. In contrast, there was a negative correlation between ACC PV+ neurons and alcohol consumption levels in females while males had a weak positive correlation. These findings suggest that PV+ neurons are not the underlying neural mechanism between prior adolescent alcohol consumption and changes in the rate of operant extinction learning. However, these findings also suggest that there is an association between PV+ neurons and adolescent alcohol consumption (possibly due to pre-existing differences in PV+ neuron levels), with sex differences. Future research is needed to determine the nature of this relationship.

Disclosures: A. Lensing: None. **R. Boerger:** None. **S. Gilbert:** None. **H. Lin:** None. **C. Pickens:** None.

Poster

P735. Alcohol: Cognitive and Behavioral Effects

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Program #/Poster #: P735.07

Topic: G.09. Drugs of Abuse and Addiction

Support: UO1 AA 028710
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P50 AA017823-S1
T32 AA 025606

Title: Sex difference and recognition impairments during social memory task in young adults following adolescent intermittent ethanol exposure

Authors: *J. P. MATO, P. T. NUNES, A. BLACKWELL, N. L. REITZ, B. KIPP, A. J. STANWISE, B. M. HITCHCOCK, J. N. MELITA, L. SAVAGE;
Psychology, State Univ. of New York, Binghamton, Binghamton, NY

Abstract: Adolescent binge drinking is known to be associated with cognitive impairments and with changes in brain structure and connectivity. Previous studies using a model of adolescent intermittent ethanol exposure (AIE) have shown a normal spatial working memory and stable activity-dependent dorsal hippocampal acetylcholine (ACh) efflux in early adulthood following AIE. However, other studies have shown that affective behaviors are altered by AIE in early adulthood. Given that ventral hippocampus (vHPC) regulates emotional and motivated behaviors, including social memory, we investigate whether male and female rats exposed to binge-like levels of ethanol (postnatal days (PD) 25 to 55) show disruption in social memory two months after AIE (PD120). Analysis of social interaction revealed a significant effect of sex: Male rats spent more time interacting with juveniles than female rats during both the acquisition phase and the recognition phase. A significant effect of recognition (more time spent with novel juvenile over familiar juvenile) was found and planned contrasts revealed that control male and control female rats (water-gavage treatment) spent more time interacting with a novel juvenile rat than with a familiar juvenile, recognizing the familiar rat. In contrast, AIE male and AIE female rats spent similar amounts of time interacting with novel or familiar juvenile rats, demonstrating impairment in social memory. To further understand the behavioral effects of AIE and whether the cholinergic response in the vHPC is associated with the disruption of social memory, social recognition task was adapted to microdialysis procedures. Acetylcholine samples were extracted via microdialysis during social recognition tasks and vHPC ACh will be analyzed via High Performance Liquid Chromatography. Also, protein levels of nicotinic and muscarine receptors, and vesicular acetylcholine transporter (VChAT) will be analyzed in brain regions involved with social memory, such as vHPC and amygdala.

Disclosures: J.P. Mato: None. **P.T. Nunes:** None. **A. Blackwell:** None. **N.L. Reitz:** None. **B. Kipp:** None. **A.J. Stanwise:** None. **B.M. Hitchcock:** None. **J.N. Melita:** None. **L. Savage:** 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.; P50 AA017823.

Poster

P735. Alcohol: Cognitive and Behavioral Effects

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Program #/Poster #: P735.08

Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA R37AA01684

Title: Male, but not female, Val68Met BDNF mice exhibit social anxiety, prefer alcohol over social interaction, and are resistant to the anxiolytic effects of alcohol

Authors: *J. J. MOFFAT[1], S. A. SAKHAI[2], D. RON[3];
[2]Neurol., [1]Univ. of California, San Francisco, San Francisco, CA; [3]Dept. of Neurol., UCSF, San Francisco, CA

Abstract: The BDNF Val66Met polymorphism, which results in impaired activity-dependent BDNF release, is prevalent in humans and associated with multiple psychiatric disorders. We previously developed an animal model of this polymorphism and showed that mice carrying the mutation (Met68BDNF mice) exhibit excessive and compulsive alcohol drinking behaviors, compared to Val68BDNF mice (1). In this study we examined behavioral deficits in Met68BDNF mice that may be linked to excessive alcohol use. We first hypothesized that Met68BDNF mice may exhibit anxiety-like behavior and consume excessive alcohol as a means of self-medication. We found that male Met68BDNF mice display distinct social anxiety-like behavior in a three-chamber social novelty paradigm, while female Met68BDNF mice and wild type Val68BDNF mice of both sexes do not. We further showed that male Met68BDNF exhibit a greater preference for alcohol compared with social interaction in a real-time place preference test. We then examined the hypothesis that Met68BDNF mice consume more alcohol than Val68BDNF mice because of an impaired response to the anxiolytic actions of alcohol. To do so, mice were systemically administered with alcohol (1.25 g/kg) or saline and anxiety-like behavior was tested using an elevated plus maze. We found that male Met68BDNF mice are less sensitive to the anxiolytic effects of alcohol compared with Val68BDNF mice. The ventral hippocampus (vHC) is a brain region linked to social anxiety-like behavior and alcohol dependence in rodents. We hypothesized that overexpressing Val68BDNF in the vHC of male Met68BDNF mice may be sufficient to improve social anxiety-like behavior and increase sensitivity to the anxiolytic effects of alcohol. Indeed, we found that overexpression of wild type Val68BDNF in the vHC of male Met68BDNF mice ameliorates social anxiety-like behavior rescues a social aversion phenotype in a social-alcohol conditioned place preference/aversion test. Overexpressing Val68BDNF in the vHC also restored the anxiolytic effect of alcohol in male Met68BDNF mice. Taken together, these data suggest that the BDNF Val68Met polymorphism causes social anxiety-like behavior in male mice and engenders a resistance to the anxiolytic effects of alcohol. These phenotypes, in turn, are likely to influence the excessive and compulsive alcohol consumption observed in these animals. 1. Warnault et al. Biological Psychiatry. 2016.

Disclosures: J.J. Moffat: None. **S.A. Sakhai:** None. **D. Ron:** None.

Poster

P735. Alcohol: Cognitive and Behavioral Effects

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Howard Hughes Medical Institute

Title: Neuromolecular and behavioral effects of ethanol deprivation in Drosophila

Authors: *K. MCCULLAR[1], N. D'SILVA[2], A. CONARD[3], T. BLACKWATER[1], R. AZANCHI[1], U. HEBERLEIN[5], E. LARSCHAN[4], K. KAUN[4];
[1]Neurosci., [2]Alpert Med. Sch., [3]Computer Sci., [4]Brown Univ., PROVIDENCE, RI; [5]Janelia Res. Campus, Howard Hughes Med. Inst., Ashburn, VA

Abstract: Alcohol use disorder (AUD) is a complex and relapsing disorder that is characterized, in part, by periods of abstinence followed by a loss of control in limiting alcohol intake. The behaviors associated with cessation of alcohol after repeated exposures, and the underlying molecular changes have yet to be fully elucidated. Using a *Drosophila melanogaster* model, we sought to explore the effects of alcohol use and subsequent deprivation on spontaneous behavior, and the underlying neuromolecular mechanisms. We present a novel repeated intermittent ethanol exposure paradigm in *Drosophila*, and quantified behavior using the machine vision software ctrax and behavior annotator JAABA. We found that multiple days of ethanol exposure followed by ethanol deprivation resulted generally in an increase in social behaviors and a decrease in locomotor behaviors. The resulting behavioral shifts were then compared to those listed in the Browsable Atlas of Behavior-Anatomy Maps to identify brain regions associated with identified behaviors. We performed RNASeq of whole head samples to identify gene expression changes correlated with the behavioral effects. This three-pronged approach allowed us to establish a framework to measure and compare behavioral, circuit, and gene expression changes. We found that the observed locomotor and social behavioral shifts were associated with a wide range of brain regions. These marked changes in behavior were also correlated largely with changes in expression of genes associated with sensory responses and neural plasticity. Interestingly, changes in social behavior persisted longer than changes in locomotor behavior and mapped to the greater mushroom body (MB) region, and areas of the brain associated with sensory responses including the anterior ventrolateral protocerebrum (AVLP), lateral horn (LH), antennal lobe (AL), prow, lobula, superior intermediate protocerebrum (SIP), flange and subesophageal zone (SEZ). These persistent behavioral responses were correlated with changes in gene expression associated with Immune Deficiency (Imd) and Toll signaling pathways, suggesting that alcohol deprivation induces changes in innate immune responses, consequently altering behavioral responses. By locally manipulating gene expression in brain regions of interest, the molecular mechanisms underlying the observable behavioral shifts associated with ethanol deprivation can be causally understood.

Disclosures: K. McCullar: None. **N. D'Silva:** None. **A. Conard:** None. **T. Blackwater:** None. **R. Azanchi:** None. **U. Heberlein:** None. **E. Larschan:** None. **K. Kaun:** None.

Poster

P755. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P755.01

Topic: G.09. Drugs of Abuse and Addiction

Support: NSERC Discovery Grant

Title: The effect of acute stressors on heroin seeking after punishment-imposed abstinence in male rats.

Authors: *J. CHARLES[1], C. BORGES[1], U. SHALEV[2];
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Abstract: Stress is a major trigger of relapse in addicts following a period of abstinence. Recently, we found that acute food-deprivation stress increases heroin seeking in rats using a punishment-imposed abstinence model. Currently, it is not known whether the effect of food deprivation on heroin seeking generalizes to other stressors following punishment-imposed abstinence. Furthermore, correlations between drug use and individual differences in traits such as exploratory drive, anxiety, reward-seeking, and pain-sensitivity have been found. In the current study, adult male Long Evans rats underwent the following pre-screening procedures: sucrose preference test (SP; a measure of reward seeking), elevated-plus maze (EPM; a measure of anxiety-like behaviour), open field test (OF; a measure of exploratory behaviour), and tail-flick test (TF; a measure of pain sensitivity). Following pre-screenings, rats were trained to self-administer heroin under a seek-take chain for 14 days. Completed seeking links were subsequently punished on 30% of trials. Next, rats were exposed to a stressor, electrical footshock, and a heroin-seeking test. A second heroin-seeking test was subsequently run after 24 hr food-deprivation stress. Exposure to footshock stress resulted in a statistically significant increase in heroin seeking. Interestingly, food-deprivation stress did not. High- and low-responders identified through median split were statistically significantly different in both the footshock- and food-deprivation-induced relapse tests. We found statistically significant differences between high- and low-responders in the stress-induced relapse tests in the following parameters: time spent freezing in the EPM (footshock), distance travelled in the OF (footshock), time in center in the OF (footshock), and fecal boli in the OF (footshock and food-deprivation). Only fecal boli in the OF significantly predicted responding during the heroin-seeking test following both footshock and food-deprivation.

Disclosures: J. Charles: None. **C. Borges:** None. **U. Shalev:** None.

Poster

P755. Opioids: Reinforcement, Seeking, and Reinstatement II

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Program #/Poster #: P755.02

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA009813
NIH Grant DA050325

Title: Glucagon-like peptide-1 receptor agonist reduces cue- and drug-induced fentanyl seeking in rats

Authors: *L. A. URBANIK[1,2], N. ACHARYA[1], P. S. GRIGSON[1];
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Abstract: Opioid Use Disorder (OUD) is a crisis in the United States today. It is a chronic relapsing disorder that has negative impacts on the individual, the family, and the community at large. In 2019, the United States recorded over 70,630 drug overdose deaths. Opioid overdose deaths accounted for nearly 70% of these deaths. According to the National Institute on Drugs of Abuse, opioid related deaths rose from 21,088 in 2010 to 49,860 in 2019. Further, the rise in opioid related deaths coincides with a significant rise in the use of the 150 times more potent synthetic opioid, fentanyl, where in 2019, there were 36,359 fentanyl overdose deaths. Since 2020, the United States has been facing yet another surge in opioid related deaths during the COVID-19 pandemic. Monthly drug overdose deaths have increased in nearly every state and, from February to May, saw an increase of over 9,000 deaths, an increase of nearly 50%. Furthermore, this overdose trend has spared no demographic and costs the nation an estimated $51.2 billion annually. Thus, it is imperative to better understand the underlying mechanisms of OUD, in order to identify potential targets for treatment. Using animal models, studies have shown that rats readily self-administer heroin and, following an extinction period, reinstate drug-seeking behavior when presented with cues for drug, the drug itself, or stress. We have shown that treatment with the glucagon-like peptide-1 receptor (GLP-1R) agonist, liraglutide, can reduce heroin seeking behavior in rats. Therefore, using our rodent model, we established a fentanyl self-administration paradigm, to test whether treatment with the GLP-1 receptor agonist also can reduce fentanyl seeking in fentanyl experienced rats. The results of the study show that rats readily self-administer fentanyl (0.5 ug/kg) intravenously with evidence of individual differences whereby some rats (High Takers) self-administer more fentanyl than others (Low Takers). As with other drugs of abuse, following a period of extinction, rats readily reinstate fentanyl seeking when challenged with cue, fentanyl and stress. Here, treatment with the GLP-1 receptor agonist, liraglutide (0.3 mg/kg sc), was found to attenuate both cue-induced fentanyl seeking and drug-induced reinstatement of fentanyl seeking at the same efficacy as the currently approved opioid agonist, buprenorphine. Taken together, these data suggest that a known satiety signal, GLP-1, may serve as an effective non-opioid alternative for the treatment of OUD without the risk of opioid dependence or precipitated withdrawal.

Disclosures: L.A. Urbanik: None. **N. Acharya:** None. **P.S. Grigson:** None.

Poster

P755. Opioids: Reinforcement, Seeking, and Reinstatement II

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Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant AA027301

Title: Sex-dependent effect of chronic intermittent ethanol vapor exposure on oxycodone self-administration in C57BL/6J mice

Authors: *Y. CHEN, A. BOISVERT, A. KIMBROUGH;
Basic Med. Sci., Purdue Univ., West Lafayette, IN

Abstract: While substance use disorder (SUD) is well studied, most preclinical research focuses on investigating the use of a single drug in isolation, whereas polysubstance use disorder is widespread in the real world. One potential mechanism for co-abuse of multiple drugs is self-medication with one drug to alleviate the withdrawal symptoms of another. We sought to design a preclinical model of self-medication of oxycodone during withdrawal from alcohol. We hypothesized that withdrawal from alcohol dependence would modulate oxycodone intake. Male (n=42) and female (n=42) C57BL/6J mice were used in this study. Mice were split into two groups, alcohol dependent oxycodone self-administration (CIE+O; n=16/sex) and oxycodone self-administration (O only; n=16/sex). The CIE+O group was made alcohol-dependent through chronic intermittent ethanol vapor (CIE) exposure. During the CIE weeks, mice were exposed to 16 hours of alcohol vapor per day, 5 days a week. Following the CIE weeks, the mice received a one week break. After three weeks of CIE exposure (week 6), the mice underwent intravenous catheterization (IV) surgery to insert catheters into their right jugular vein. The mice then received a fourth week of CIE exposure and then the following week were tested for IV self-administration of oxycodone (0.25 mg/kg/infusion) for 4 hours per day for 5 days. During this week, the O only group also self-administered oxycodone daily. Both O only and CIE+O mice then paused oxycodone self-administration while the CIE+O group received the fifth week of CIE. Following this week of CIE, both O only and CIE+O mice resumed oxycodone self-administration for 4 days. Brains were collected 24 hours after the last self-administration session for further examination.A significant increase in self-administration of oxycodone was found in the last three sessions in the male CIE+O mice compared to O only mice. However, female CIE+O mice did not show the same increase in oxycodone self-administration compared to O only mice. These data suggest that we can model the use of oxycodone to self-medicate alcohol withdrawal in mice. Additionally, males appear to be more sensitive to modulation of oxycodone intake by alcohol withdrawal; however, this may be due to a ceiling effect in the

female mice. Further testing will be necessary to determine the specific mechanisms behind the sex differences. We are currently examining neural activity patterns across the brain in each group to explore potential treatment or sex effects.

Disclosures: Y. Chen: None. **A. Boisvert:** None. **A. Kimbrough:** None.

Poster

P755. Opioids: Reinforcement, Seeking, and Reinstatement II

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA049139 (RTL)
NIH Grant DA048055 (RTL)

Title: Infralimbic pyramidal neuron activity after an unreinforced lever press is necessary for encoding extinction of heroin seeking in rats

Authors: *M. S. MCGREGOR[1], K. E. NETT[1], A. R. ZIMBELMAN[2], R. T. LALUMIERE[1,2,3]; [1]Interdisciplinary Grad. Program in Neurosci., [2]Dept. of Psychological and Brain Sci., [3]Iowa Neurosci. Inst., Univ. of Iowa, Iowa City, IA

Abstract: The medial prefrontal cortex (mPFC) has been implicated in the regulation of heroin-seeking behavior. Our laboratory has previously published data indicating that activity in the infralimbic (IL) portion of the mPFC following an unreinforced lever press is critical for encoding the extinction of cocaine seeking. For heroin seeking, however, evidence on the role of the IL has been mixed. Some work suggests that the IL suppresses cued reinstatement to heroin seeking. Yet other evidence suggests that the IL promotes reinstatement of heroin seeking in some situations. Thus, it is unclear whether the extinction process itself recruits the same mechanisms for cocaine and heroin seeking. To determine whether IL activity is similarly necessary for extinction of heroin seeking, we employed an activity-controlled optogenetic approach to inhibit IL pyramidal neurons for 20 s immediately after unreinforced lever presses during extinction training. Male and female Sprague-Dawley rats received bilateral injections of AAV-CaMKII-eNpHR3.0 into the IL and bilateral optical fibers above the IL. Rats then underwent 12 d of 6 h operant heroin self-administration training, where active lever presses produced a .075 mg/kg heroin infusion, light/tone cues, and lever retraction. These experiments utilized an availability window design, where the active lever was only available for 90 s windows throughout a session. Then, rats proceeded with 5 d of 2 h extinction sessions, where active lever presses only produced lever retraction and 20 s of laser illumination to the IL to inhibit pyramidal neurons during the period immediately following an unreinforced lever press. This was followed by 7 d of unmanipulated extinction sessions, where lever presses resulted only

in lever retraction, which served as an index of retention of extinction learning. Preliminary results indicate that inhibiting IL activity for 20 s after an unreinforced lever press increases lever pressing in male rats during the initial 5 d of extinction training, suggesting impaired encoding of the extinction learning. Moreover, rats that received inhibition also had impaired retention of extinction learning, as indicated by higher levels of lever pressing during the 7 d of unmanipulated extinction sessions. Ongoing work is examining whether this is also the case in female rats. Nonetheless, this finding indicates that IL activity in the 20 s following an unreinforced lever press is critical for encoding the lever press-no reward contingency that facilitates extinction of heroin seeking, suggesting that IL activity is similarly important for encoding extinction of both cocaine and heroin seeking.

Disclosures: M.S. McGregor: None. **K.E. Nett:** None. **A.R. Zimbelman:** None. **R.T. LaLumiere:** None.

Poster

P755. Opioids: Reinforcement, Seeking, and Reinstatement II

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant GM083883

Title: Effects of repeated fentanyl treatment on the locomotor activity of adolescent and adult rats

Authors: D. Y. LOPEZ SANCHEZ, J. W. RIOS, H. A. DIAZ, J. A. TAYLOR, D. J. GONZALEZ, *C. A. CRAWFORD;
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Abstract: Opioid misuse has reached epidemic proportions in the United States, primarily as a result of the increased availability of synthetic prescription opioids like fentanyl. Despite the growing problem of fentanyl abuse, few preclinical investigations have assessed the addictive properties of this compound. The goal of the current study was to assess the abuse liability of fentanyl using a multi-trial behavioral sensitization paradigm. In this experiment, adolescent and adult male and female Sprague-Dawley rats (*n*=9-10) were given daily injections of saline or fentanyl (100 or 200 µg/kg, sc) for seven consecutive days. Immediately after injections, rats were placed in locomotor activity chambers for 60 min. After a 2 or 10 day abstinence period, all rats were injected with fentanyl (100 µg/kg, sc) and locomotor activity was measured for 120 min. On the first pretreatment day, fentanyl (100 and 200 µg/kg) reduced the locomotor activity of adolescent and adult male and female rats. However, by pretreatment day seven, the suppressive effects of fentanyl were no longer apparent and all rats treated with 100 µg/kg

fentanyl exhibited more locomotor activity than rats treated with saline. On both test days, fentanyl (100 and 200 µg/kg) pretreatment resulted in more locomotor activity than saline pretreatment, but some sex- and age-dependent differences were apparent. After two abstinence days, adolescent female rats were more active than adolescent male rats. Adult rats did not exhibit any sex differences, but adults pretreated with 200 µg/kg fentanyl were more active than adults pretreated with 100 µg/kg fentanyl. After ten abstinence days, pretreatment with 200 µg/kg fentanyl caused more locomotor activity in male and female adolescent rats than 100 µg/kg fentanyl. Interestingly, adolescent rats showed an increased sensitivity to fentanyl after ten abstinence days, while adult rats had similar levels of locomotor activity on both test days. In summary, our results suggest that both adolescent and adult rats showed a progressively enhanced locomotor response after repeated fentanyl exposure. Moreover, adolescent rats were more sensitive to fentanyl after a 10 day, as compared to a two day, abstinence period.

Disclosures: D.Y. Lopez Sanchez: None. **J.W. Rios:** None. **H.A. Diaz:** None. **J.A. Taylor:** None. **D.J. Gonzalez:** None. **C.A. Crawford:** None.

Poster

P755. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P755.06

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01DA046532
Welch Foundation Grant AQ-0039

Title: Interactions between fentanyl and 2,5-dimethoxy-4-methylamphetamine (DOM), a direct-acting 5-HT_{2A} receptor agonist, in rhesus monkeys responding under a food versus drug choice procedure

Authors: *D. R. MAGUIRE, C. P. FRANCE;
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Abstract: Direct-acting 5-HT_{2A} receptor agonists such as DOM enhance antinociceptive effects of opioids suggesting a 5-HT_{2A} receptor agonist could be combined with an opioid to improve treatment of pain. However, it is unclear whether 5-HT_{2A} receptor agonists also enhance adverse effects of opioids, including reinforcing effects that contribute to abuse. This study examined whether DOM alters the reinforcing effects of fentanyl in rhesus monkeys (n=6) responding under a food versus drug choice procedure. Responding on one lever delivered sucrose pellets and responding on the other lever delivered intravenous infusions. Sessions comprised 4 blocks, each with 2 forced trials, during which only one option was available, followed by 6 choice trials, during which both options were available. The unit dose of fentanyl (0.0001-0.0032

mg/kg/infusion) increased across blocks each session. In the first experiment, sessions were preceded by intravenous administration of DOM (0032-0.32 mg/kg), naltrexone (0.032 mg/kg), or heroin (0.1 mg/kg). Then, fentanyl was available for self-administration in combination with DOM in unit dose ratios (fentanyl to DOM) of 1:3 or 1:10. Fentanyl increased choice of infusions from less than 10% of trials with the two smallest unit doses to over 80% of trials with the two larger unit doses. Naltrexone decreased choice of fentanyl, shifting the dose-effect curve rightward, and heroin increased choice of the two smallest unit doses of fentanyl. When administered before the session, DOM reduced choice of fentanyl as well as the number of trials completed. However, DOM did not alter choice or the number of trials completed when available in combination with fentanyl. Attenuation of fentanyl choice by naltrexone and enhancement of fentanyl choice by heroin demonstrate sensitivity of responding to modulation by other drugs. DOM did not appear to enhance the reinforcing effects of fentanyl whether given as a pretreatment or made available in combination with fentanyl. Although pretreatment with DOM decreased choice of fentanyl, it did so at doses that reduced trials completed, suggesting effects of DOM might have been due, at least in part, to generalized rate-decreasing effects. Taken together with previous studies, these results suggest that DOM enhances some (antinociceptive) but not other (reinforcing) effects of opioids and that combining a direct-acting 5-HT_{2A} receptor agonist with an opioid to treat pain would not increase potential for abuse.

Disclosures: D.R. Maguire: None. **C.P. France:** None.

Poster

P755. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P755.07

Topic: G.09. Drugs of Abuse and Addiction

Support: R01 DA047443
Boettcher Foundation Webb-Waring Award

Title: Ventral Tegmental Area Glutamate Neurons Contribute to Cue-Induced Oxycodone Seeking Behavior

Authors: *D. MCGOVERN[1], D. H. ROOT[1], A. M. POLTER[2], E. PREVOST[1], A. LY[1], D. MULCAHY[1];
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Abstract: Ventral tegmental area (VTA) GABA neurons regulate dopamine neuron activity via a local projection modulated by the mu-opioid receptor. A subset of neurons within the VTA, defined by the presence of the vesicular glutamate transporter (VGluT2), also regulate dopamine

neuron activity via a local projection but their role in drug-seeking behavior is unknown. We first identified that select subsets of VTA VGluT2+ neurons express the mu opioid receptor. Further, optogenetic activation of local projections from VTA VGluT2 neurons results in mu-opioid receptor sensitive currents. These data led us to hypothesize that VTA VGluT2 neurons participate in opioid-seeking behavior. To test this hypothesis, we leveraged an oral oxycodone self-administration paradigm to determine the functional role of VGluT2+ VTA neurons in both oxycodone self-administration and cue-induced drug-seeking during reinstatement. Male and female VGluT2::Cre mice were surgically injected with a FLEX-GFP (control) or FLEX-hM4Di AAV in the medial ventral tegmental area prior to oxycodone self-administration. After four weeks mice learned to operantly self-administer escalating doses of oxycodone solution by nose-poking an illuminated port that also resulted in a tone-light off cue. Nose pokes into the inactive port were recorded but had no programmed consequence. At the highest dose (1 mg/mL) mice were given an IP injection of behaviorally-subthreshold clozapine to activate the hM4Di DREADD and silence VTA glutamate neuron activity in the experimental group. Mice self-administered for three additional days at this dose following hM4Di activation before transitioning to extinction. During the extinction phase mice were placed in the same operant box for one hour with illuminated nose ports, however no behavioral action produced either the drug paired cue or the availability of oxycodone. Following two consecutive sessions of active pokes below extinction criterion, mice were tested for cued reinstatement in the presence of hM4Di activation or control conditions (GFP). Chemogenetic inactivation of VTA VGluT2+ neurons did not significantly modify drug consumption at the highest oxycodone dose, however silencing these neurons during cued reinstatement significantly reduced cue-induced oxycodone seeking compared to GFP control mice. This work establishes a functional role for VTA glutamate neurons in prescription opioid cued reinstatement behavior.

Disclosures: D. McGovern: None. **D.H. Root:** None. **A.M. Polter:** None. **E. Prevost:** None. **A. Ly:** None. **D. Mulcahy:** None.

Poster

P755. Opioids: Reinforcement, Seeking, and Reinstatement II

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Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P755.08

Topic: G.09. Drugs of Abuse and Addiction

Support: Brain Research Foundation
Center for Compulsive Behavior & Addiction

Title: Attenuation of cue-induced heroin-seeking by the atypical antidepressant mirtazapine

Authors: *A. L. PERSONS, T. C. NAPIER;
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Abstract: Opioid use disorders (OUD) and associated mortality have emerged in recent years as a significant public health problem in many countries. Opioids activate *mu*-opioid receptors, and currently available pharmacotherapies for OUD all target this receptor as a full agonist (methadone), a partial agonist (buprenorphine), or an antagonist (naltrexone). These medications promote recovery, but relapse to drug-taking remains high. The addiction profile of opioids reflects imbalances in downstream transmitters, including dopamine and serotonin (5-HT). The 5-HT_{2A} and 5-HT_{2C} receptors (R) bidirectionally regulate dopamine release. The atypical antidepressant mirtazapine has multiple sites of action, including agonism at 5-HT_{2A}R and inverse agonism at the 5-HT_{2C}R. This favorable 5-$HT_{2A/2C}$R profile suggests it may be efficacious for OUD therapy. We previously demonstrated that mirtazapine reduces stimulant-induced behaviors in rats (Herrold *et al*, 2009; Voigt *et al*, 2011; Voigt & Napier, 2011), including drug-seeking behavior in rats that self-administered methamphetamine (Graves & Napier, 2011) and opioid-induced place preference (Graves *et al*, 2012). Here, we hypothesized that mirtazapine will attenuate cue-induced heroin-seeking behavior. Young adult male Sprague-Dawley rats were trained to nose-poke for heroin (0.075mg/kg per 1mL infusion) on a fixed ratio-1 schedule of reinforcement for 10 days (6h/day). One day after heroin self-administration (SA), a baseline cue reactivity test (CR, 20min) was conducted. Thereafter, rats were subjected to an intermittent SA /CR cycle in which rats self-administered heroin for two days, followed by a CR test the following day; this cycle was repeated until each rat was tested for CR in the presence of vehicle (1mL/kg, ip), mirtazapine (1mg/kg, ip) or mirtazapine (5mg/kg, ip) injected 30min prior to the test. A one-way repeated measures ANOVA with *post hoc* Newman-Keuls was used to analyze the number of active nose-pokes during the CR session. Mirtazapine at 1mg/kg had no effect on heroin-seeking. In contrast, 5mg/kg mirtazapine significantly decreased heroin-seeking ($p<0.05$). These positive outcomes suggest that mirtazapine has the potential to be repurposed for relapse reduction during abstinence therapy of OUD.

Disclosures: A.L. Persons: None. **T.C. Napier:** None.

Poster

P755. Opioids: Reinforcement, Seeking, and Reinstatement II

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P755.09

Topic: G.09. Drugs of Abuse and Addiction

Support: NSERC Discovery grant

Title: Neuronal activity during acute food deprivation-induced heroin seeking test, after punishment-imposed abstinence, in male rats.

Authors: *C. BORGES, E. AH YEN, J. CHARLES, B. GIULIANO, U. SHALEV;
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Abstract: Punishment-imposed abstinence in animal procedures models self-imposed abstinence due to negative consequences of drug-use in addicts. Understanding the mechanisms that underlie self-imposed abstinence due to negative consequences is crucial to better understand human abstinence. Stress is one of the key triggers in human relapse. Environmental stressors such as food deprivation are recurrent in addicts who chose to allocate their money towards drugs over food. Caloric restriction is suggested to be positively correlated with increase cravings and subsequent relapse in humans abstaining from cigarettes and alcohol. Thus, the goal of this study is to identify the brain areas that are involved in food deprivation-induced relapse to heroin seeking after punishment-imposed abstinence. Rats (N=30) underwent heroin self-administration training (SA, 0.1 mg/kg/infusion) for 2 weeks using the seek-take chain (completing a VI60 schedule of reinforcement on the seek lever allowed access the drug-paired lever - take lever, under FR1). Next, rats were exposed to 4 days of punishment-imposed abstinence, in which 30% of the completed seek links resulted in a mild footshock instead of access to the take lever. The footshock (0.5 s) gradually increased at 0.1 mA/day from 0.2 to 0.4 mA and remained fixed at 0.4 mA for one more day. For heroin-seeking tests, rats were assigned to 24 h food deprivation (FD) or Sated groups and tested under extinction conditions (1 h). The following groups were used: 1. Rats only exposed to SA (Baseline, N=6), 2. Rats exposed to SA and punishment (N=4), 3. Rats exposed to SA, punishment and FD/sated conditions without test (FD: N=3, Sated: N=3) and 4. Rats exposed to SA, punishment, FD/Sated conditions and heroin-seeking test (FD: N=7, Sated: N=7). All brains were collected after the test session or at the same time of the day. Fos expression is currently assessed through immunohistochemistry, while focusing on brain areas related to stress (central nucleus of the amygdala (CeA), basolateral amygdala (BLA), bed nucleus of the stria terminalis (BNST)), reward (prefrontal cortex (PFC), ventral tegmental area (VTA), nucleus accumbens (NAc)), feeding and conflict (paraventricular nucleus of the thalamus (PVT), ventral hippocampus (vHPC)).

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Poster

P755. Opioids: Reinforcement, Seeking, and Reinstatement II

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Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P755.10

Topic: G.09. Drugs of Abuse and Addiction

Support: DA049130

Title: Withdrawal from oxycodone self-administration increases cocaine intake

Authors: *S. KHATRI[1], H. ULANGKAYA[3], W. STOOPS[2], C. GIPSON-REICHARDT[1]; [1]Family and Community Med., [2]3.Department of Behavioral Science, Psychiatry and

Psychology, Ctr. on Drug and Alcohol Res., Univ. of Kentucky, Lexington, KY; [3]Barrett Honors Col., Arizona State Univ., Tempe, AZ

Abstract: Opioid use disorder (OUD) is a leading public health crisis in the United States. Individuals with OUD frequently use other substances concurrently, including cocaine. Opioid withdrawal has been linked to increased cocaine use motivation, although the neurobiology underlying this pattern of co-use is as of yet unknown. Thus, the aim of this study is to develop a preclinical opioid and cocaine co-use model in rats to further evaluate underlying mechanisms. Male and female Long Evans rats underwent sequential self-administration (SA) of the μ opioid agonist, oxycodone (0.03 mg/kg/infusion; FR-1), followed by cocaine SA (0.25 mg/kg/infusion; FR-1) sessions beginning in acute oxycodone withdrawal (16 hrs after the final oxycodone session) in an A-B-A-B design. Rats underwent a minimum of 10 oxycodone SA ("A") sessions prior to the first cocaine SA ("B") phase, followed by oxycodone SA ("A") and a day of cocaine SA ("B", test day). Somatic signs of opioid withdrawal were measured prior to initiating the first cocaine session and during each switch from oxycodone to cocaine SA. Control rats self-administered food followed by cocaine. Rats readily discriminated between the active (food/oxycodone) and inactive levers. Further, the average number of cocaine infusions and consumption on test day was higher in the oxycodone compared to food SA group, and there was an increasing trend in the total number of oxycodone infusions with switch back to oxycodone after cocaine SA. No sex specific differences were observed in cocaine intake. Somatic signs (e.g., foot licks, grooming, among others) were increased at 16 to 20hrs of oxycodone withdrawal that were attenuated by subsequent cocaine SA. These results suggest that oxycodone withdrawal increases cocaine SA. In all, this study establishes the first model of this pattern of polysubstance use which can be further used to study underlying neurobiology.

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Poster

P761. Opioids: Addiction Circuits

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Program #/Poster #: P761.01

Topic: G.09. Drugs of Abuse and Addiction

Support: UG3DA050325
R37DA009815
NIH RF1MH12460501
5TL1TR002016-04
1S10OD018124-01A1 (TA)

Title: The effect of first-time heroin exposure on c-Fos signaling in rodentsThe effect of first-time heroin exposure on c-Fos signaling in rodents

Authors: *B. EVANS, S. BALLARD, K. NEWMASTER, Y. KIM, S. GRIGSON;
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Abstract: While we are familiar with brain areas and pathways implicated in opioid use disorder (OUD), we do not have a full understanding of the neural circuits activated upon first drug exposure. In order to identify areas of the brain most activated by the first exposure to opioids, transgenic c-Fos-GFP mice were injected interperitoneally (ip) with saline or 10mg/kg heroin and returned to their home cages. Two hours later, the mice were transcardially perfused with 1x phosphate buffered saline (PBS) followed by 4% paraformaldehyde (PFA) in PBS. Brains were removed and post-fixed in 4% PFA at 4℃. They were placed in 1% agarose mold in order to be prepared for two photon microscopy imaging. The results showed a trending increase in c-Fos activation following exposure to heroin. Specifically, we observed c-Fos activation differences in the Nucleus Accumbens (NAc), Lateral Hypothalamus (LH), Orbitofrontal Cortex (OFC), Basolateral Amygdala (BLA), Habenula, Nucleus Tractus Solitarus (NTS), Parabrachial Nucleus (PBN), Periaqueductal Gray (PAG), Cingulate Cortex (CC), and Locus Coeruleus (LC). We looked at these brain regions in particular, because they are known to be associated with reward, satiety, or aversion. We also examined c-Fos based cortex activation in rats by utilizing a tissue clearing and 3D immunolabeling method combined with light sheet microscopy. Naïve male Sprague Dawley rats were injected with glucagon-like peptide-1 receptor agonist (GLP-1RA), 0.3mg/kg liraglutide, or saline 6 hours before injection with 10mg/kg heroin. Two hours after heroin injection, the rats were transcardially perfused with 1x phosphate buffered saline with sodium azide (PBS/NaN_3) followed by 4% paraformaldehyde (PFA) in PBS. Brains were removed, post-fixed as described, delipidated, immunolabeled with c-Fos, and cleared for light sheet microscopy imaging. Here, treatment with the GLP-1RA reduced heroin-induced c-Fos activation in the cortex in rats - providing a mechanism by which such treatment also reduces heroin intake, cue-induced seeking, and drug-induced reinstatement.

Disclosures: B. Evans: None. **S. Ballard:** None. **K. Newmaster:** None. **Y. Kim:** None. **S. Grigson:** None.

Poster

P761. Opioids: Addiction Circuits

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Program #/Poster #: P761.02

Topic: G.09. Drugs of Abuse and Addiction

Support: 5R01DA011289-21
5T32DA035165-08

Title: Characterizing supraspinal pain-relevant glutamatergic afferents to the Ventral Tegmental Area.

Authors: *C. MANNING[1], J. A. KAUER[2];
[1]Stanford Univ., West Menlo Park, CA; [2]Dept. of Psychiatry, Stanford Univ., Stanford, CA

Abstract: Many ventral tegmental area dopamine neurons (VTA_{DA}) exhibit reduced firing rates in response to injury or noxious events (Ungless et al, 2004, Science 303: 2040; Huang et al, 2019, Mol.Brain 12:89), and disinhibition of VTA_{DA} neurons results in analgesia, indicating that VTA firing contributes to behavioral responses to noxious or aversive events. However, VTA_{DA} synaptic physiology in response to injury has not been extensively characterized. The VTA is innervated by regions implicated in the control of pain, including the periaqueductal grey (PAG), anterior cortex, and lateral habenula -- all of which send glutamatergic afferents to VTA_{DA}. To characterize these circuits after injury we isolated specific glutamatergic afferents to identified dopaminergic neurons (PITX3-GFP), and compared their function in saline- and carrageenan-treated mice. To achieve afferent-type specificity, we injected AAV-DJ-hSyn-ChR2-eYFP at p21, and 3 weeks later recorded optically-evoked EPSCs. We pursued the PAG-VTA_{DA} afferent, as PAG has a known role in opioid analgesia and its excitatory afferents to the VTA can drive aversion. Paired pulse ratios in response to various interstimulus intervals (50ms, 100ms, 500ms, 1000ms) were unchanged between inflammatory injury and saline-treated animals (N=20 cells, 11 animals, p=0.11). Furthermore, no differences were seen between groups comparing the first to the tenth EPSC in an optically evoked train of 10 stimuli at 1, 5, 10, or 20Hz under either basal or inflammation states (N=16 cells/ 11 animals, p=0.18). Our data suggest that probability of release at excitatory PAG terminals does not change in our inflammation model. We have begun to characterize postsynaptic responses of the PAG-VTA_{DA} synapses. Intriguingly, many of these VTA_{DA} neurons displayed excitatory currents at -70 mV that were not blocked by NBQX (10uM), as well as excitatory currents at +40 not blocked by d-AP5 (50uM). We therefore tested the GluA2-lacking AMPAR antagonist, NASPM, as well as the NR2C/D receptor antagonist, UBP141. Many optically evoked PAG-VTA_{DA} EPSCs were decreased by UBP141(24uM) and NASPM (20uM), indicating the presence of both NR2C/D NMDARs and GluA2-lacking AMPA receptors at these synapses. We are currently testing whether acute inflammation alters AMPA/NMDA ratios as well as sensitivity to NASPM and UBP141. Our data show that presynaptic function in PAG-VTA_{DA} synapses appears unchanged 24 hours after carrageenan injection, and preliminary results suggest no difference in overall AMPA/NMDA ratios following inflammatory injury. Potential avenues for future study include excitatory PAG afferents on VTA GABAergic neurons.

Disclosures: C. Manning: None. **J.A. Kauer:** None.

Poster

P761. Opioids: Addiction Circuits

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Program #/Poster #: P761.03

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA R01 DA039895
NSF DGE-1848739
McManus Foundation

Title: Chronic morphine increases expression of the neuropeptide NMS in ventral tegmental area dopamine neurons

Authors: *C. M. RIVERA-QUILES, S. C. SIMMONS, A. L. GARRISON, N. M. CAMACHO-FONTÁNEZ, M. S. MAZEI-ROBISON;
Michigan State Univ., East Lansing, MI

Abstract: Although opioid dependence and addiction continue to constitute a major health and economic burden, our limited understanding of the underlying neurobiology limits better diagnostics and interventions. Dysregulation of the mesocorticolimbic reward circuit is acknowledged to contribute to various aspects of drug addiction, with alteration in the activity and output of dopamine (DA) neurons in the ventral tegmental area (VTA) known to contribute to the rewarding aspects of drug use. However, the molecular mechanisms underlying these changes in VTA DA function remain relatively unexplored. Thus, we used translating ribosome affinity purification (TRAP) to identify gene expression changes in mice that specifically occur in VTA DA neurons following chronic morphine exposure. We have validated gene expression changes via qPCR and intriguingly, we find that expression of a number of neuropeptides not traditionally described in the VTA are robustly induced by morphine exposure. Neuromedin S (NMS) was of particular interest as it was enriched in VTA DA neurons and its expression was robustly increased following chronic morphine exposure. It is known that the primary receptor with which NMS interacts, neuromedin U receptor 2 (NMUR2), is expressed within the target regions of VTA DA neurons, such as the nucleus accumbens (NAc). However, whether all VTA DA neurons express NMS, and their potential functional impact has yet to be determined. Thus, we have begun to characterize the expression and functional impact of VTA DA neurons that co-express NMS using NMS-Cre mice and Cre-dependent viral vectors. We have found that a subset of VTA DA neurons express NMS (<5%) and that these neurons exhibit diverse projection targets. Using DREADDs, we have found that acute activation of VTA-NMS neurons does not affect general locomotor activity or elicit conditioned place preference or aversion. We are now assessing whether activation of VTA-NMS neurons alters morphine-elicited behaviors (conditioned place preference, locomotor sensitization). Together, these studies are expected to set the stage for future work investigating the role of specific VTA-DA^{NMS} circuits, their activity during behavior, and their potential as targets for therapeutic intervention.

Disclosures: C.M. Rivera-Quiles: None. **S.C. Simmons:** None. **A.L. Garrison:** None. **N.M. Camacho-Fontánez:** None. **M.S. Mazei-Robison:** None.

Poster

P761. Opioids: Addiction Circuits

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Program #/Poster #: P761.04

Topic: G.09. Drugs of Abuse and Addiction

Support: NS027881
DA034748

Title: Subtype specific changes in the intrinsic electrophysiological properties of mouse hypocretin/orexin (H/O) neurons following exposure to chronic morphine

Authors: *E. BERRY[1], S. JAVED[1], R. MCGREGOR[2], C. WALLER[1], C. HOLLANDER[1], M. ISHIBASHI[3], J. M. SIEGEL[2], C. S. LEONARD[1];
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Abstract: Hypothalamic H/O neurons exhibit structural and functional plasticity in their regulation of arousal, feeding, and reward. Indeed, human heroin addicts have H/O somata that are ~20% smaller and 50% more numerous than those in control subjects (Thannickal et al. 2018). Our NEURON simulations of this size change in simplified H/O-neurons (soma + 5 dendrites) indicate that the resulting altered passive properties increase their excitability. We therefore investigated the impact of chronic morphine on H/O-EGFP neurons by whole-cell recordings in acute brain slices from male mice (9-18 w) treated with a single daily injection (s.c.) of either morphine (50 mg/kg) or saline for 14 days. With investigators blind to the treatment, we confirmed that somatic cross-sectional area of H/O-EGFP neurons was ~17% smaller in morphine (n=4, ~100 neurons/mouse) vs. saline (n = 4, ~90 neurons/mouse; $p < 0.05$) treated mice. We next distinguished D- and H-type H/O-EGFP neurons by their membrane responses which are dominated by A-type K^+ currents in H-cells (Schöne et al. 2011). Morphine treatment did not alter the proportion of D- or H- type neurons (saline n = 18 D /13 H cells; morphine n = 17 D /14 H cells) or alter their spontaneous firing, sEPSC amplitudes, input resistance or cell capacitance, although there was a trend toward increased capacitance and decreased sEPSC amplitudes for H cells ($p = 0.07$). However, morphine treatment selectively altered spiking in H cells, for which rheobase was increased by ~43%, action potential amplitude was decreased by ~10% and the peak afterhyperpolarization was ~3 mV less negative (t-test, $p < 0.05$) compared to H-cells from saline treated mice. Repetitive firing in response to 2s constant current pulses was also selectively decreased with the F-I curve down-shifted following morphine treatment in H- but not D- cells. Moreover, firing patterns for H-, but not D-cells shifted from one of continuous firing toward more clustered and reluctant firing during the current pulse. Since structural plasticity is reversed after four weeks of withdrawal (Thannickal et al. 2018), we are testing whether these changes in H-cell excitability are also reversed

following withdrawal. In summary, our data indicate that in addition to inducing structural plasticity in H/O neurons, chronic morphine induces an intrinsic plasticity of H-type neurons which decreases their excitability. This implies that H- and D-type H/O neurons differentially participate in brain reward pathways and addiction, and raises the possibility that selective impairment of information outflow from H-cells may contribute to the hypodopaminergic state associated with chronic opiate use.

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Poster

P761. Opioids: Addiction Circuits

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Program #/Poster #: P761.05

Topic:

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T32 MH112507

Title: μ-opioid receptor signaling bias alters opioid-induced dopamine release in the mesolimbic circuit

Authors: *S. W. GOODING[1], C. CHAU[2], E. LEWIS[3], J. L. WHISTLER[3];
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Abstract: Disinhibition of midbrain dopamine neurons is a well-studied action of opioids in the brain. The μ-opioid receptor (MOR), an inhibitory GPCR found on GABAergic terminals in the ventral tegmental area (VTA), reduces tonic GABA release when bound by agonists. MOR signaling to G-protein is titrated by arrestin-3 in the presence of endogenous MOR agonists. In contrast, most clinical opioids, such as morphine, produce G-protein signaling with little arrestin-3 engagement and are therefore “biased” for G-protein compared to endogenous ligands. We have previously shown that both analgesia and reward (CPP) by morphine are potentiated in knock-in mice (RMOR) with a MOR that effectively recruits arrestin-3 when activated by morphine. Furthermore, RMOR mice are resistant to development of analgesic tolerance and physical dependence following repeated morphine administration. This suggests that balanced signaling at the MOR is effective in combating both short and long-term homeostatic responses to MOR signaling such as receptor desensitization and upregulation of cAMP.We hypothesized that signaling bias at the MOR would affect the opioid-sensitive mesolimbic circuit and modulate downstream dopamine release. Using the fluorescent sensor dLight1.3b we recorded dopamine release in medial or lateral shell of the nucleus accumbens during intravenous opioid

administration in both WT and RMOR mice. Our data show differences in morphine-induced dopamine release between the two genotypes indicating that signaling bias produces circuit-level effects that are measurable downstream of the VTA. We further explore this hypothesis with methadone, a clinically important MOR agonist that recruits arrestin-3, to further implicate signaling bias at the MOR as a key presynaptic modulator of striatal dopamine release.

Disclosures: S.W. Gooding: None. **C. Chau:** None. **E. Lewis:** None. **J.L. Whistler:** None.

Poster

P761. Opioids: Addiction Circuits

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P761.06

Topic: G.09. Drugs of Abuse and Addiction

Support: LDRD 20-ERD-009

Title: Neuron-glial co-cultures exhibit differential molecular and functional signatures following sub-chronic exposure to low and high doses of fentanyl

Authors: D. LAM, A. SEBASTIAN, C. BOGGURI, S. K. G. PETERS, M. E. MENDEZ, N. R. HUM, C. A. VALDEZ, N. O. FISCHER, G. G. LOOTS, *H. A. ENRIGHT;
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Abstract: Fentanyl is one of the most frequently used synthetic opioids in medicine where it has established itself as an analgesic for patients undergoing surgery, and in the proper management of chronic pain. Unfortunately, due to its euphoric side effects, illicit use has been dramatically increasing, contributing to a steep rise in lethal drug overdoses. Fentanyl, like morphine, binds to mu-opioid receptors (MOR) in many brain regions, and this binding event can lead to addiction, tolerance, impairment of cognitive functions, and inhibit nociception, arousal, and respiration. Much research has focused on opioid receptor tolerance and the effector mechanisms at a cellular level, but how fentanyl affects the neural activity at a system level is poorly understood. Using a neuron-glial brain-on-a-chip system developed by our lab, we examined the neuroadaptive molecular and functional neural network changes following sub-chronic exposure to fentanyl. Primary rat co-cultures, containing cortical neurons, astrocytes and oligodendrocyte precursor cells, were seeded on a multi-electrode array (MEA) or 96-well tissue culture plate (for serial endpoint bulk RNA sequencing and imaging analyses). Once networks matured (28 days *in vitro*), co-cultures were treated with a low (0.01 μM) or high (10 μM) dose of fentanyl, and electrophysiology was monitored daily for 5 days. Sub-chronic fentanyl exposure resulted in a decline in neural (i.e., spiking) and network (i.e., bursting) activity, with diminished activity sustained for 5 days at the high dose condition. For the low dose condition, a decline at 24 hours relative to vehicle control was observed. Cultures treated with the mu-opioid receptor (MOR)

antagonist CTOP, in the presence of fentanyl, revealed that diminished activity was MOR-dependent in the low-dose condition but MOR-independent in the high-dose condition. The MOR-dependent and independent effect induced by low and high doses, respectively, paralleled pre-and postsynaptic gene expression changes predominantly observed in the low-dose condition, while other biological processes (e.g., neurotransmitter synthesis, growth factor expression and cytokine signaling) were found altered in cultures exposed to the high dose. Collectively, the findings of this study suggest that neuroadaptive changes to neural network activity at a systems level *in vitro* is dependent on the concentration of fentanyl, where at a low dose, fentanyl exposure primarily influences neuronal activity while at a high dose both neurons and glial cells are affected.

Disclosures: D. Lam: None. **A. Sebastian:** None. **C. Bogguri:** None. **S.K.G. Peters:** None. **M.E. Mendez:** None. **N.R. Hum:** None. **C.A. Valdez:** None. **N.O. Fischer:** None. **G.G. Loots:** None. **H.A. Enright:** None.

Poster

P761. Opioids: Addiction Circuits

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Program #/Poster #: P761.07

Topic: G.09. Drugs of Abuse and Addiction

Support: NSF GRFP
OHSU startup funds

Title: Calcium-permeable AMPA receptors are upregulated in the rat nucleus accumbens during incubation of oxycodone craving

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Abstract: The prescription opioid oxycodone is a widely prescribed and highly effective treatment for pain, but its addicting properties and severe withdrawal symptoms have contributed significantly to the opioid epidemic. Avoiding relapse to oxycodone during abstinence is a major challenge for recovering oxycodone users in part because cues associated with previous oxycodone intake can elicit cravings that often lead to relapse even after prolonged periods of abstinence. Identifying synaptic mechanisms underlying heightened cue-induced cravings during abstinence is critical for developing appropriate treatments for opioid use disorder. Cue-induced opioid cravings that persist throughout abstinence can be studied in rats using the incubation of drug craving model, in which cue-induced drug seeking progressively increases during withdrawal from drug self-administration. Studies of incubation of stimulant craving (cocaine,

meth) have demonstrated that upregulation of Ca^{2+}-permeable AMPA receptors (CP-AMPAR) in the nucleus accumbens (NAc) core mediates expression of incubation in late withdrawal. Here we determined whether upregulation of CP-AMPAR also occurs in parallel with incubation of oxycodone craving. Male and female Long Evans adult rats underwent 10 days of extended access oxycodone or saline self-administration (6h/d for 10 d; 0.1mg/kg/infusion; infusion paired with light cue). Rats received a 30 min test for cue-induced drug seeking on withdrawal day 1 (WD1) during which responding in the previously active nose poke hole delivered the cue in the absence of drug, and another 'seeking test' on either WD15 or WD30. Two to 12 days after the WD15 seeking test, whole cell recordings of medium spiny neurons in the NAc core were performed. The contribution of CP-AMPAR to the excitatory postsynaptic current was measured in oxycodone and saline groups using the rectification index (RI). Rats showed significant increases in cue-induced responding on WD15 vs WD1, indicating expression of incubation of craving (the WD30 study is in progress). There were no observed sex differences in drug intake or incubation. Compared to saline cells, oxycodone cells showed an elevated RI, indicating upregulation of CP-AMPAR in the NAc core between WD15 and WD30. These results suggest that CP-AMPAR upregulation is a form of plasticity common to protracted withdrawal from stimulants and the opioid oxycodone. Thus, preventing or reversing CP-AMPAR upregulation in the NAc during withdrawal may be a potential strategy for preventing cravings and relapse to multiple different classes of addicting drugs.

Disclosures: K.A. Engeln: None. **E. Hwang:** None. **M.E. Wolf:** None.

Poster

P761. Opioids: Addiction Circuits

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Program #/Poster #: P761.08

Topic: G.09. Drugs of Abuse and Addiction

Support: DA049140
GM123582
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HHMI

Title: Opioid Biosensors for Pharmacokinetics: Organelles to Whole Animals

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Abstract: The chronic effects of exogenous opioids originate in part from intracellular interactions. In addition to plasma membrane (PM) activation, some opioids bind to their receptors in the endoplasmic reticulum (ER) to facilitate trafficking via pharmacological chaperoning and in Golgi/endosomes to effect signaling. At the whole animal scale, physiological and genomic variance and drug delivery mode lead to varied pharmacokinetics, complicating behavioral analyses. To provide a basis for cellular and circuit mechanisms of opioid action, we optically measure pharmacokinetics across these spatial scales. We developed a new class of genetically encoded biosensors, "intensity-based opioid-sensing fluorescent reporters", or "iOpioidSnFRs" to detect fentanyl, methadone, and naltrexone among other drugs. We adapted a biosensor scaffold based on OpuBC, a choline-binding periplasmic binding protein, and circularly permuted GFP. We screened 16 biosensor mutants against 40+ opioids representing μ, δ, and κ ligands to discover initial hits. Through directed evolution, we converted OpuBC variants to opioid binding proteins. Isothermal titration calorimetry and stopped-flow kinetics fleshed out a biophysical model for the new biosensors. These biosensors fulfill key criteria: (1) sensitivity across the pharmacologically relevant concentration range, (2) selectivity against endogenous ligands and other drugs, (3) sufficient reporter kinetics, and (4) photostability during all desired experiments. Results: (A) We established culture assays for organellar pharmacokinetics. Biosensors were directed to the PM, cytoplasm, ER, and Golgi using address sequences. Transfected HeLa cells and transduced primary neurons were imaged under perfusion across a range of [opioid]. Opioids permeated the ER nearly as well as they access the PM, indicating that ample drug is available for chaperoning. Furthermore, methadone accumulated in the Golgi ~4-fold relative to the extracellular solution, supporting the receptor activation observed in that compartment. (B) We recorded cytoplasm-directed iFentanylSnFR in freely behaving mice via fiber photometry. AAV9-hSyn-iFentanylSnFR was injected in the VTA and used to detect fentanyl after IP injection. These measurements are synchronized with annotated video to provide a dose-dependent behavioral time course. This method addresses: (1) whether animals self-administer to optimize a certain [opioid] vs. time waveform and (2) the *individualized* behavioral hallmarks at the heights and troughs of [opioid], where we expect possible euphoria and onset of withdrawal respectively.

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Poster

P761. Opioids: Addiction Circuits

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH / NIDA Grant U01 DA051993
Hennepin Healthcare Research Institute Career Development Award

Title: Evaluation of morphine withdrawal-induced anhedonia in male and female rats

Authors: *A. C. HARRIS[1], P. MUELKEN[1], J. R. SMETHELLS[1], S. X. LIU[2], A. RAMAKRISHNAN[3], L. SHEN[3], P. TRAN[4], M. G. LESAGE[1], J. C. GEWIRTZ[2];
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Abstract: Understanding mechanisms underlying individual differences in susceptibility to opioid addiction is essential to developing more effective, individualized treatments. A major challenge in studying such mechanisms in preclinical models is the lack of a reliable behavioral predictor of future opioid self-administration. We recently reported that elevations in intracranial-self stimulation (ICSS) thresholds (anhedonia-like behavior) during withdrawal from acute morphine in male rats was negatively correlated with a wide range of measures of subsequent i.v. morphine self-administration. These findings implicate withdrawal-induced anhedonia (WIA) as a reliable prospective behavioral marker of individual differences in opioid addiction vulnerability. The goal of this study was to characterize WIA under a more clinically relevant morphine dosing regimen involving multiple spontaneous withdrawal episodes rather than a single spontaneous withdrawal episode, and to provide the first characterization of WIA in females. Repeated daily injections of morphine (5.6 mg/kg, s.c) produced a progressive increase in ICSS thresholds (i.e., WIA) measured 23 hr after each injection in both sexes, whereas repeated saline injections did not affect ICSS thresholds in either sex. There was no significant sex difference in the magnitude of WIA. The degree of WIA showed considerable individual variability (e.g., range of ICSS thresholds during final 3 test sessions (% baseline) = 100.3%-139.3%) - sufficent to allow for evaluation of the relationship between individual differences in magnitude of WIA and gene expression in future studies. Consequently, we are now combining this behavioral measure with next-generation sequencing (NGS) techniques to characterize alterations in the transcriptome and epigenome in the medial prefrontal cortex (mPFC) of rats tested for WIA. Based on our recent study using a similar morphine dosing regimen without WIA testing, we expect to observe significant up-regulation of genes relevant to synaptic signaling and neuroplasticity in the mPFC of both sexes. These findings establish a protocol for evaluating individual differences in the early stages, and escalating severity, of the anhedonic component of opioid withdrawal in both sexes. Integration of these behavioral data with NGS outcomes promises to yield novel molecular targets underlying individual differences in vulnerability to opioid addiction.

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Poster

P847. Learning and Memory: Molecular Mechanisms I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P847.01

Topic: H.08. Learning and Memory

Title: Novel role for reverse transcriptase in Aplysia synaptic facilitation

Authors: *A. R. GOLD[1], D. L. GLANZMAN[2,3];
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Abstract: Most research on learning and memory focuses on changes in synaptic strength as a mnemonic mechanism; however a growing body of evidence has emerged in recent years that indicates that nuclear mechanisms play a central role in memory storage. Studies investigating nuclear phenomena associated with memory have focused primarily on epigenetic changes, for example, DNA (de)methylation and/or histone post-translational modification patterns; but retrotransposon activity has also been implicated in memory storage. Many transposable elements (TEs) operate through an RNA intermediate; these TEs therefore require the enzyme reverse transcriptase, the sequence for which is embedded in the transposon genetic sequence itself. To investigate the potential role of TEs in long-term memory in *Aplysia*, we tested whether reverse transcriptase activity was required for long-term facilitation (LTF) in *Aplysia* sensorimotor cocultures. As shown previously, exposing *Aplysia* sensorimotor cocultures to five spaced bouts of serotonin (5HT) induces facilitation of the *in vitro* sensorimotor synapse that persists for 24-72 h. This form of synaptic facilitation represents the cellular analog of long-term sensitization of the defensive withdrawal reflex in the intact animal. By contrast, a single pulse of 5HT induces short-term facilitation (STF) of the *in vitro* sensorimotor synapse, which lasts for < 30 min and corresponds to short-term sensitization of the withdrawal reflex. We found that the reverse transcriptase inhibitor lamivudine blocked 5HT-induced LTF in *Aplysia* sensorimotor cocultures. Unexpectedly, lamivudine also partially blocked STF of *in vitro* synapses produced by a single pulse of 5HT. Lamivudine by itself did not affect the strength of sensorimotor synapses, thereby indicating that the lamivudine-induced blockade of 5HT-induced plasticity was not due to detrimental or nonspecific effects of the drug on the neurons' health. Our data reveal novel roles for reverse transcriptase in both LTF and STF in *Aplysia*. They indicate that genetic rearrangement may occur during long-term memory in *Aplysia*. In addition, the effect of lamivudine on STF suggests an intriguing potential role for reverse transcriptase in short-term memory.

Disclosures: A.R. Gold: None. **D.L. Glanzman:** None.

Poster

P847. Learning and Memory: Molecular Mechanisms I

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Topic: H.08. Learning and Memory

Support: Canadian Institutes of Health Research (CIHR)
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Title: Effect of type 1 diabetes on capillary obstructions and cognitive function in mice

Authors: *S. SHARMA, K. A. TENNANT, C. BROWN;
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Abstract: Introduction Recent work from our lab has shown that the brain capillaries routinely get clogged by cells and debris even under healthy conditions. The present study was undertaken to determine how experimentally induced type 1 diabetes affects this phenomenon, and whether obstructions contribute to cognitive decline. **Methods** C57BL/6 male and female mice were injected with streptozotocin to induce type 1 diabetes. These mice were implanted with cranial windows and cortical volumes were repeatedly imaged from 3-9 weeks after induction of diabetes. To model susceptibilities to short or long lived obstructions, we injected (i.v.) 5µm diameter fluorescent microspheres in diabetic and control mice at 30 minutes and 3 days before euthanasia. The density of microsphere obstructed capillaries was quantified across 15 different brain regions. To determine the impact of diabetes on cognitive and sensorimotor activity, mice were subjected to a battery of behavioural tests. **Results** 2-photon imaging indicated that diabetic mice have higher rates of capillary stalling in somatosensory cortex that became more pronounced with duration of diabetes. The majority of stalls in diabetic mice were associated with Rhod6G labelled cells, suggesting that leukocytes play a key role. Increased stalling also led to greater pruning of cortical capillaries. Consistent with these observations, our fluorescent microsphere obstruction assay yielded significantly higher levels of short and long lived capillary obstructions in both male and female diabetic mice, including those treated with insulin. Behaviourally, diabetic mice were not different from controls in tests of anxiety or ambulatory activity (open field or elevated plus maze), nor did they show any differences in visual function. However, diabetic mice were significantly impaired in learning/memory tests such as the novel object recognition, water maze learning or reversal learning. **Conclusions** These studies suggests that diabetes is associated with greater risk for capillary obstructions in the brain as well as learning/memory deficits. Our future aims will provide a mechanistic understanding of how diabetes elevates one’s susceptibility to capillary obstructions and cognitive decline, and whether manipulating certain immune signalling pathways can alleviate these impairments.

Disclosures: S. Sharma: None. **K.A. Tennant:** None. **C. Brown:** None.

Poster

P847. Learning and Memory: Molecular Mechanisms I

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Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P847.03

Topic: H.08. Learning and Memory

Support: NWO Rubicon
Niels Stensen fellowship

Title: Disruption of memory allocation by stress: a role for parvalbumin interneurons and endocannabinoid signaling

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Abstract: AIMS: The ability to remember which cues in a particular environment predict threat, and which do not, is imperative for survival. Overgeneralization of threatening cues, such that also neutral cues are perceived as threatening, may result in excessive anxiety-like behavior, and is a core symptom in many anxiety-related disorders and PTSD. Stress may promote such overgeneralization of memory, and hence may instigate or amplify anxiety-disorders. The overarching objective of these studies is to decipher the key pathways that underlie stress-induced memory generalization at the level of behavior, network dynamics and the cellular level, with a focus on inhibitory interneuron signaling.
METHODS: Mice were exposed to corticosterone injections or acute restraint stress, and the specificity of memory during fear conditioning and reward learning was assessed. Using *in vivo* fiber photometry, opto- and chemogenetics and iDISCO we establish the underlying cell types and networks mediating these effects.
RESULTS: After stress, mice show stronger memory generalization, both for memory with a positive and negative valence. In parallel, the lateral amygdala ensembles encoding for these memories are enlarged, and these enlarged ensembles were responsible for the generalized expression of the memories. Stress suppresses the activity of parvalbumin $(PV)^+$-interneurons, and indeed the effects of stress on memory generalization and ensemble size could be prevented by activation of PV^+-interneurons, while inhibition of PV^+-interneurons mimicked the effects of stress. We next show that endocannabinoid signaling within the lateral amygdala, which is directly modulated by stress, underlies the stress effects on interneurons and memory generalization.
CONCLUSIONS: Stress results in generalized memory expression, and disrupted memory allocation, which is prevented by modulating PV^+-interneuron activity. We propose that endocannabinoid signaling mediates these effects. Understanding these neurobiological

mechanisms of stress-induced memory generalization may be a first step towards the development of novel treatment strategies for anxiety-related pathologies.

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Poster

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Program #/Poster #: P847.04

Topic: H.08. Learning and Memory

Support: U01DA04163205

Title: C57bl/6 substrain differences in conditioned fear and hippocampal transcriptome

Authors: L. R. GOLDBERG, A. SEBASTIAN, I. ALBERT, ***T. GOULD**;
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Abstract: Accumulating evidence supports significant phenotypic and genomic differences between the closely related C57BL/6 (B6) substrains, C57BL/6J (B6J) and C57BL/6N (B6N). Inconsistent differences in conditioned fear between B6J and B6N have been reported. These inconsistences may be due to B6N substrain selection (B6NJ vs B6NCrl) or fear conditioning paradigm characteristics. Here, we aimed to determine if B6J and B6NJ substrains differed in contextual fear conditioning and learning-induced hippocampal transcriptome. Male B6J and B6NJ (PND60, n=8 per group) were fear conditioned with 1 pairing, 2 pairings, or 4 pairings of footshock and auditory cue, and then were tested for hippocampus-dependent contextual fear learning or hippocampus-independent cued fear learning. A 2-way ANOVA of Strain and number of Pairings for contextual fear learning revealed a significant effect of Strain ($F_{1,41}$=48.83, $p<0.001$), a significant effect of Pairings ($F_{2,41}$=4.99, $p<0.05$), but no interaction of Strain and Pairings. For all number of Pairings, B6NJ showed enhanced contextual freezing compared to B6J. A 2-way ANOVA of Strain and number of Pairings for cued fear learning revealed a significant effect of Strain ($F_{1,41}$=5.46, $p<0.05$), but no significant effect of Pairings or interaction of Strain and Pairings. For all number of Pairings, B6NJ showed enhanced cued freezing compared to B6J. To investigate baseline and learning-induced changes in hippocampal transcriptome that may underlie the observed behavioral differences between B6J and B6NJ, dorsal (DH) and ventral hippocampi (VH) were dissected for RNAsequencing. Samples were collected from B6J and B6NJ male mice from three groups (n=6 per group): direct from home cage, 30 minutes after 2-pairings of shock and cue, 30 minutes after 2 exposures to cue alone. Preliminary analyses of B6J and B6NJ home cage transcript level expression identified 336 DH and 153 VH differentially expressed transcripts. Enrichment analyses using Enrichr identified 7

enriched GO Biological Processes (adjusted p-value <0.05), including regulation of synapse maturation, glutamatergic synaptic transmission, and regulation of nervous system development. Continued analyses include learning-induced differential transcriptome expression between B6J and B6NJ in both hippocampal regions. Together, these behavioral and transcriptomic findings confirm differences in conditioned fear between the commonly used B6 substrains, B6J and B6NJ, and begin to uncover the underlying hippocampal transcriptome differences.

Disclosures: L.R. Goldberg: None. **A. Sebastian:** None. **I. Albert:** None. **T. Gould:** None.

Poster

P847. Learning and Memory: Molecular Mechanisms I

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Topic: H.08. Learning and Memory

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DoD PRCRP Horizon Award W81XWH-19-1-0594 to AN

Title: Neuronal Nsun2 deficiency causes epitranscriptomic dysregulation of Gly-tRNAs and proteomic shifts impacting synaptic function and behavior

Authors: *J. BLAZE[1], C. J. BROWNE[3], H. L. PHILLIPS[4], A. NAVICKAS[6], S. HEISSEL[7], A. PLAZA-JENNINGS[1], Z. T. PENNINGTON[8], D. J. CAI[9], H. MOLINA[7], T. PAN[10], B. JAVIDFAR[11], H. GOODARZI[6], W.-D. YAO[5], F. G. HAGHIGHI[2], E. J. NESTLER[13], S. AKBARIAN[12];
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Abstract: Protein translation is a critical component of brain function, and aberrant translational processes have been implicated in psychiatric disease. Transfer (t)RNAs, non-coding clover-leaf shaped RNAs that are key players in ribosomal protein synthesis, are barely explored in brain. Nsun2, a mammalian tRNA cytosine methyltransferase, is expressed at high levels in brain and

has been linked to neurodevelopmental defects in humans and mice. Previous work has shown that loss of tRNA cytosine methylation produces deficits in protein synthesis in non-neuronal cells, but the role of tRNA methylation in adult neuronal function has not been explored. We used conditional ablation and transgene-derived overexpression of Nsun2 in the mouse prefrontal cortex (PFC) to manipulate tRNA methylation levels postnatally. We used targeted RNA bisulfite sequencing and tRNA sequencing to assess tRNA methylation/expression, LS-MS/MS to measure protein expression, electrophysiological recordings to assess synaptic transmission, and behavioral testing for complex behaviors including cognition, behavioral despair, and addiction-related behaviors. Results showed that complex behaviors relating to emotion, cognition, and addiction are highly sensitive to bi-directional changes in Nsun2 in prefrontal cortex (PFC) neurons. Further, Nsun2-deficient cortex showed a selective deficit in multiple glycine tRNAs which resulted in codon-specific shifts in the proteomic landscape, with decreases in glycine-rich neuronal proteins that were associated with impaired neurotransmission and altered behavioral phenotypes. Overall, we found that tRNA methylation is a critical process for regulating synaptic plasticity and behavior through proteomic changes in the mature cortex. Additionally, this data suggests that there is another mechanism, aside from glycinergic receptors, by which glycine could critically regulate brain function and complex behaviors, suggesting potential for novel therapeutic avenues in psychiatry.

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Poster

P847. Learning and Memory: Molecular Mechanisms I

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Topic: H.08. Learning and Memory

Support: NIH P20GM103423
Orphan Disease Center's MDBR Pilot Grant Program

Title: Tet2 demethylation of DNA after learning destabilizes memory

Authors: *M. M. CARON, M. M. BISHOP, S. M. STRUGAR, B. G. MALACHOWSKY, K. E. ZENGELER, C. P. GETTENS, H. P. SMITH, A. J. KENNEDY;
Bates Col., Lewiston, ME

Abstract: Active DNA methylation in neurons that encode experience is required for long-term memory formation. TET enzymes oxidize and lead to the removal of DNA cytosine methylation.

Here we demonstrate that the conditional knockout of Tet2 in glutamatergic neurons induces enhanced long-term memory retention in mice. We found enhanced DNA methylation patterns in the promoter of experience-regulated genes due to Tet2 deletion in these neurons, which corresponded with altered gene expression. Additionally, we demonstrate that deleting Tet2 from recently activated neurons, induced by learning, selectively enhanced the recall of individual memories. These data suggest that Tet2 is a negative regulator of memory retention in neurons following learning and a target for memory enhancement.

Disclosures: M.M. Caron: None. **M.M. Bishop:** None. **S.M. Strugar:** None. **B.G. Malachowsky:** None. **K.E. Zengeler:** None. **C.P. Gettens:** None. **H.P. Smith:** None. **A.J. Kennedy:** None.

Poster

P847. Learning and Memory: Molecular Mechanisms I

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P847.07

Topic: H.08. Learning and Memory

Support: NIH Grant MH107254
NIH Grant MH057014

Title: Regulation of gene expression in neurons by bivalent chromatin marks

Authors: *C. GREER[1], A. DUNCAN[2], J. WRIGHT[2], J. WEISS[1], R. J. COLBRAN[2], G. KAAS[1];
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Abstract: We recently reported that two isoforms of the Ten-eleven translocation 1 (*Tet1*) gene are transcribed in the mouse brain.[1] The longer isoform facilitates, and the shorter isoform suppresses, long-term memory formation in adult male mice. The longer isoform is less abundant, but strongly influences neuronal gene transcription and other functions. To identify mechanisms regulating *Tet1* expression, we examined the post-translational chromatin marks surrounding the two *Tet1* gene promoters from publicly-available cell-type specific chromatin immunoprecipitation sequencing data. The long isoform promoter region is co-enriched with both active (Histone 3 lysine 4 trimethylation, H3K4me3) and suppressive (Histone 3 lysine 27 trimethylation, H3K27me3) marks in neurons. The enzymes regulating these two marks are known to be important for long-term memory formation. While both the short and long promoters had H3K4me3 marks in neurons and non-neurons, only the neuronal long promoter also had H3K27me3 marks. Dually edited genomic loci with active and suppressive marks are known as bivalent chromatin in embryonic stem cells. They usually resolve themselves to either solely active or suppressive monovalent marks in differentiated cells, and have not been

previously characterized in mature neurons. However, surprisingly, an examination of bivalent chromatin derived from hippocampal neurons of adult mice revealed that hundreds of gene promoters exist in this cell-type specific bivalent state. In addition, many of these same promoters were found to be bivalently marked in neurons from the anterior cingulate cortex. Many of the neuronal bivalent genes encode transcription factors and cell-surface receptors that are involved in regulating cell proliferation, developmental pathways, and signal transduction. Specific examples include the autism-associated gene Reelin (*Reln*), and *Erbb4*, which is a schizophrenia risk gene associated with memory formation. Enhancer of zeste homolog 2 (EZH2) generates H3K27 methylation marks, and we find that EZH2 inhibitors elicited derepression of bivalently marked genes in neuronal, but not in glial cultures. Collectively, our data suggests that neurons utilize bivalent chromatin to sustain tight control over transcriptional levels for critically important genes.
[1]Greer et al., 2021. J Neurosci **41**: 578-593. PMC7842754.

Disclosures: C. Greer: None. **A. Duncan:** None. **J. Wright:** None. **J. Weiss:** None. **R.J. Colbran:** None. **G. Kaas:** None.

Poster

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Program #/Poster #: P847.08

Topic: H.08. Learning and Memory

Support: NIH-NINDS K12NS098482
UCI ICTS Pilot Project Grant
UCI Department of Pediatrics
UCI Conte Center Seed Grant
RWJF Amos Medical Faculty Development Program

Title: Paired genomic and epigenetic sequencing reveals neuronal signatures of early-life exercise

Authors: *A. S. IVY[1,2,5], A. M. RAUS[3], T. D. FULLER[4], N. E. NELSON[3], D. VALIENTES[4], A. BAYAT[4];
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Abstract: Aerobic exercise is a potent environmental signal that is associated with improved hippocampal memory. Studies in rodents have shown that neurobiological mechanisms activated by exercise include epigenetic modifications, which act to influence expression of memory-

associated genes. Less is known regarding the effects of early-life exercise on adolescent gene expression and epigenetic regulation that may influence long-term improvements in neuronal function. Traditional *in vivo* approaches to next-generation sequencing for answering these questions use heterogeneous cell populations from whole tissue homogenates. In order to evaluate neuron-specific epigenetic and transcriptional changes resulting from early-life exercise, we developed a line of transgenic mice expressing the NuTRAP cassette under the neuron specific Emx1 promoter (named Emx-NuTRAP mouse). This approach allows for simultaneous isolation of both nuclei and translating ribosomal mRNA from a primarily neuronal population. We employ a new technique that combines the nuclear isolation using Isolation of Nuclei TAgged in Specific Cell Types (INTACT) and Translating Ribosomal Affinity Purification (TRAP) methods in order to couple neuron-specific epigenetic modifications and gene expression programs driven by early-life exercise. Data from RNA-seq and CUT&RUN-seq were paired to evaluate histone modifications influencing gene expression after exercise. We also performed separate INTACT and TRAP isolations from a second group of exercised and sedentary Emx-NuTRAP mice, and show similar patterns of gene expression and histone modification occupancy. Finally, as traditional approaches sequence from opposite hemispheres in order to obtain transcriptomic and epigenomic data, we take a bioinformatics approach to determine if hemispheric differences in gene expression and histone modifications in hippocampal neurons. Our data reveal a transcriptional and epigenetic signature of early-life exercise exposure, and discovers candidate gene-histone modification interactions for further investigation. Importantly, our novel approach of combined INTACT/TRAP methods from the same cell suspension allows for simultaneous transcriptomic and epigenomic sequencing in a cell-type specific manner.

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Poster

P847. Learning and Memory: Molecular Mechanisms I

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Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P847.09

Topic: H.08. Learning and Memory

Support: LECOM seed Grant, 2020

Title: Neuroprotective Role of Integrin Linked Kinase in Prenatal Brain Development

Authors: B. LITTLE[1], N. SUD[1], M. ESCOBAR[2], *D. BHATTACHARYA[1];
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Abstract: Integrin Linked Kinase (ILK) is a synaptic scaffolding protein that plays an important role in learning and memory and glutamatergic synapse development. We previously reported using fetal alcohol spectrum disorder (FASD) rat model, the moderate alcohol exposure results in the deficits in hippocampal dependent contextual memory and synaptic plasticity in the offspring that correlate to the impaired function of ILK. ILK can be activated through stimulation of Brain Derived Neurotrophic Factor (BDNF) receptor, Tyrosine kinase B (TrkB). We have evidence to suggest that administration of 7,8 dihydroxyflavone (7,8-DHF), potent TrkB agonist attenuates spatial memory deficits in the same FASD rat model. Chronic administration of 7,8 DHF during preadolescence (P15-30) resulted in increased general activity and exploration in a Y-maze task, thus reducing some aspects of the spatial deficits otherwise observed in FASD rats. However, it is not yet tested if ILK activity is essential during brain development to counter the teratogenic effect of alcohol. Here, we evaluated the effect of intraperitoneal injection of ILK inhibitor, QLT0267 (Inh) or control saline (con) in time pregnant rats (injected on alternate days during gestation) on the spatial memory development in the offspring. Administration of QLT0267 (5mg/kg) during gestation significantly reduced the percentage dwell time in the offspring using a novel arm exploration protocol in a Y-maze compared to the control rat offspring. A Mann-Whitney test indicated the percentage dwell time was greater in con (N=11) as compared to inh (N=10) offsprings (U=23, p=0.02). This suggests that ILK activity impairment during brain development by prenatal alcohol exposure may result in deficits of spatial memory function in the offspring. There is no significant change in hippocampal ILK protein expression. ILK inhibition during early brain development has significantly reduced the (N-Methyl-D-aspartic acid) NMDA receptor subunits, NR2B (con=5, inh=4, t=2.712, df=7, p=0.03) and NR1 expression (con=5, inh=4, t=3.969, df=7, p=0.005) in the hippocampal protein lysates. However, no significant changes are observed in the expression of other glutamate receptor subunits including (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) AMPA receptors. This study concludes that ILK activity deficits dysregulated hippocampal NMDA receptor expression impairing spatial learning and memory in rats exposed to prenatal inhibition. Therefore, prenatal alcohol mediated impairment of ILK activity during development is one of many mechanisms contributing to hippocampal learning and memory deficits.

Disclosures: B. Little: None. **N. Sud:** None. **M. Escobar:** None. **D. Bhattacharya:** None.

Poster

P847. Learning and Memory: Molecular Mechanisms I

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Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P847.10

Topic: H.12. Aging and Development

Support: NIH-R37HD083217

Title: Neural circuitry for infant attachment: mother enhances hippocampal activity during early life

Authors: *R. M. ZANCA[1,2], W. SONG[3], M. OPENDAK[1,2], N. NAEEM[3], N. GRAF[3], A. I. BLOMKVIST[4], P. A. SERRANO[5], D. A. WILSON[1,2], R. M. SULLIVAN[1,2];
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Abstract: The infant attachment literature suggests that the child's attachment quality to the caregiver promotes a healthy foundation for neurobehavioral developments. Yet our understanding of the neurobiology of this relationship remains elusive. Within this relationship, one infant behavior reliably associated with the attachment figure is proximity seeking, with strangers much less likely to evoke approach. For our approach, we used infant rats because similarly to children, attachment is learned and once learned, evokes prosocial behaviors towards the attachment figure. While children rely on all sensory systems to identify the attachment figure, rat pups primarily use the mother's odor, which pups rapidly learn. Since the rat maternal odor is diet-dependent, we produced a "stranger" mother by feeding it a different diet, which pups had not learned to identify as the maternal odor. Here we define neurobehavioral responses to the mother vs. a stranger mother focusing on the hippocampus and cortex. We used 12 to 18-day old infant rat pups, an age range of robust attachment. Pups' behavior were recorded during a 1hr session with either a anesthetized mother or an anesthetized stranger mother (different maternal odor) or a no odor control (pup placed in empty arena). Sessions were videotaped and scored offline for behavioral assessment. Two measures of dorsal hippocampal activity were used, autoradiography (14C 2-DG) and western blot for synaptic D1 dopaminergic receptors (D1R) since previous has highlighted maternal control of infant rats' amygdala dopamine. Cortical and hippocampal local field potential (LFP) oscillations were also measured. Results indicate that pups spent more time with the mother and exhibited significantly more prosocial behaviors vs. the stranger. Neural markers showed greater hippocampal activity (2-DG), and increased synaptic D1R expression to the mother vs. the stranger. LFP recordings show divergent LFP responses to the mother and stranger. These results begin to better define specialized attachment circuitry within two divergent social context.

Disclosures: R.M. Zanca: None. **W. Song:** None. **M. Opendak:** None. **N. Naeem:** None. **N. Graf:** None. **A.I. Blomkvist:** None. **P.A. Serrano:** None. **D.A. Wilson:** None. **R.M. Sullivan:** None.

Poster

P864. Learning, Memory, and Spatial Navigation: Rodent Models

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P864.01

Topic: H.09. Spatial Navigation

Support: R00AG049090
R01AG070094
Florida Department of Health 044081 to AW

Title: Spatial disorientation and Alzheimer's disease: the relationship between task-induced activation profiles in the brain's navigational system and reorientation deficits in the 3xTg-AD mouse model of tau and amyloid beta aggregation

Authors: *J. R. DIXON, A. C. STIMMELL, S. C. MOSELEY, A. A. WILBER;
Dept. of Psychology, Florida State Univ., Tallahassee, FL

Abstract: Impaired navigation is one of the first symptoms of Alzheimer's disease (AD). Understanding the brain changes associated with this early impairment is critical for the development of earlier AD diagnosis methods and treatments. Previously, we found that 6-month female 3xTg-AD mice are impaired at using distal cues to get re-oriented in space (spatial reorientation task; Stimmell et al., 2019), and subsequently, that the pTau density profile across a parietal-hippocampal network was a strong predictor of this impairment (Stimmell et al., 2021). Thus, in 6-month female 3xTg-AD mice and age matched controls, we assessed the c-Fos activation profile across the parietal-hippocampal network induced by the spatial reorientation task. Specifically, we used c-Fos immunohistochemical staining to assess the c-Fos positive cell density in seven regions: the parietal cortex (PC), the dorsal and ventral CA1 field of the hippocampus, the dorsal and ventral subiculum (Sub), and the dorsal and ventral areas of the retrosplenial cortex (RSC). We found that, as in our previous work, female 6-month 3xTg-AD mice, which accumulate tau and amyloid beta (Aβ), are impaired at the spatial reorientation task (Stimmell et al., 2019). Further, task-induced c-Fos density was higher in the PC but not the remaining 6 regions, suggesting that there may be hyperactivation of the parietal cortex of 3xTg-AD mice. This is consistent with some studies that have reported hyperactivation of the default mode network in individuals with preclinical AD (Sperling et al., 2009) and of the cortex of AD mice in response to Aβ oligomers (Keskin et al., 2017). Surprisingly, the strongest correlations between task induced c-Fos activation and spatial reorientation performance were with the CA1 and RSC and the weakest correlations were with the ventral Sub and PC. Taken together, this suggests that network level dysfunction is driving the hyperactivation in PC and that other components of this network are more closely tied to spatial reorientation performance. Finally, the direction of the strongest correlations was negative, meaning that the higher the density of activation in the RSC and the CA1 region of the hippocampus, the worse spatial learning and memory (i.e., more active RSC and CA1 cells were associated with worse performance). This suggests that, at early time points prior to significant tau and Aβ accumulation (e.g., pre-plaque), hyperactivity in the parietal-hippocampal network may be reflective of pathophysiology of AD.

Disclosures: J.R. Dixon: None. **A.C. Stimmell:** None. **S.C. Moseley:** None. **A.A. Wilber:** None.

Poster

P864. Learning, Memory, and Spatial Navigation: Rodent Models

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Program #/Poster #: P864.02

Topic: H.09. Spatial Navigation

Support: NIA R00 AG049090
R01AG070094
Florida Department of Health 044081

Title: Sleep Reverses Spatial Reorientation Impairments in a Mouse Model of Alzheimer's Disease

Authors: *A. C. STIMMELL, J. R. DIXON, S. C. MOSELEY, A. A. WILBER;
Florida State Univ., Tallahassee, FL

Abstract: In early Alzheimer's disease (AD), getting lost is one of the first cognitive impairments to emerge. The precise cause of this impairment is unclear. Previously, we demonstrated that impaired spatial navigation may result from a failure to use distal cues to get oriented in space (spatial reorientation task; Stimmell et al., 2019). This impaired use of distal cues for spatial reorientation in 3xTg-AD mice, which accumulate both amyloid beta (Aβ) and tau, emerges early in disease progression before the onset of plaques and tangles, suggesting the impairments we observed in mice may be informative about those observed in early AD in humans (Henderson et al., 1989; Weintraub & Salmon, 2012; Allison et al., 2016). Only 6-month female 3xTg-AD (not 3-month female) mice were impaired on the spatial reorientation task. In addition, we have also shown that the profile of phosphorylated tau positive cell density across brain regions associated with spatial navigation is highly predictive of spatial learning and memory performance in 6-month female 3xTg-AD mice (Stimmell et al., 2021). The search for treatments of AD is ongoing and some treatments are aimed at prolonging quality of life or staving off early symptoms of AD. One such approach is modifying sleep. Poor sleep quality, short sleep duration, and disrupted slow wave sleep are all associated with increased Aβ, poor cognition, and risk of AD (Nebel et al., 2018). Decreased sleep is associated with an increase in the release of tau and phosphorylated forms of tau seen in the earliest stages of AD, as well as decreased clearance of tau (Lucey 2020). Sleeping is also associated with Aβ and tau clearance (Lucey 2020; Nebel et al., 2018; Tononi & Cirelli 2014; Toro et al., 2019). Thus, we set out to test the hypothesis that sleep could ameliorate impairments we observed in 6-month female 3xTg-AD mice. We assessed spatial reorientation performance either with or without pre- and post-task sleep sessions. We replicated our previously observed spatial reorientation impairment in no-sleep mice; while sleep mice did not differ from age-matched controls. Thus, sleep modification may ameliorate cognitive dysfunction in AD.

Disclosures: A.C. Stimmell: None. **J.R. Dixon:** None. **S.C. Moseley:** None. **A.A. Wilber:** None.

Poster

P864. Learning, Memory, and Spatial Navigation: Rodent Models

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Program #/Poster #: P864.03

Topic: H.09. Spatial Navigation

Support: NIA R00 AG049090
R01 AG070094
Department of Health 044081

Title: Rescuing impaired cortical-hippocampal interactions during sleep in a mouse model of Alzheimer's disease

Authors: S. D. BENTHEM[1], I. SKELIN[3], S. MOSELEY[1], J. DIXON[1], A. STIMMELL[1], A. A. WILBER[2];
[1]Psychology, [2]Florida State Univ., Tallahassee, FL; [3]Neurosci., Univ. of Lethbridge, Lethbridge, AB, Canada

Abstract: In preclinical Alzheimer's disease (AD), spatial learning and memory is impaired (Allison et al., 2016). We reported similar navigational impairments in 6-month 3xTg-AD female mice on a virtual task that requires learning and memory for using landmarks to get oriented in space (Benthem et al., 2020). Memory replay during sleep is critical for learning related plasticity (Ego-Stengel & Wilson, 2009; Jadhav et al, 2012; Maingret et al, 2016), and hippocampal-cortical dysfunction is a potential mechanism for memory impairments in individuals with AD (Gennaro et al, 2017; Khan et al, 2014).We found that early in disease progression deficits in hippocampal-parietal cortex (PC) coordination during sleep coincided with navigation impairments on the virtual maze (Benthem et al, 2020). Gamma-stimulation (40Hz) has been shown to clear AD pathology in mice (Iaccarino et al, 2016; Martorell et al, 2019), and improve functional connectivity in preclinical AD patients (He et al, 2021). Thus, we assessed hippocampal-PC coordination in 6-month female 3xTg-AD/PV^{cre} (4xTg) mice that were learning the same *spatial reorientation task*. Mice were implanted with a 16 tetrode recording array targeting PC and hippocampus and an optical fiber aimed at HPC. Then daily recording sessions of rest-task-rest commenced as mice learned to locate the unmarked reward zone. During the same surgery a cre-dependent AAV was used to express channel rhodopsin 2 in the parvalbumin+ interneurons in the hippocampus. This allowed for daily stimulation sessions, with either 40Hz entrainment in hippocampus or SHAM stimulation. We assessed sleep quality metrics, multi-unit activity patterns, cortical spindles, and delta waves (DW) in PC and markers of memory replay in the hippocampus (SWRs) during SWS. We found no difference in any sleep

quality metrics. We also found no difference in number or rate of DW or SWRs. However, in SHAM stimulated mice were SWR-DW cross-correlations were significantly reduced, similar to our previous findings in 3xTg-AD mice (Benthem et al, 2020). In the 40Hz stimulated mice, this functional connectivity was rescued. Furthermore, the 40Hz stimulated mice showed improved performance on the virtual reorientation task compared to the SHAM stimulated mice. Thus, 40Hz stimulation of hippocampus may rescue functional connectivity impairments in the hippocampal-PC network and spatial navigation.

Disclosures: S.D. Benthem: None. **I. Skelin:** None. **S. Moseley:** None. **J. Dixon:** None. **A. Stimmell:** None. **A.A. Wilber:** None.

Poster

P864. Learning, Memory, and Spatial Navigation: Rodent Models

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Program #/Poster #: P864.04

Topic: H.09. Spatial Navigation

Support: University of San Diego Faculty Research Grant
Ronald McNair Program
University of San Diego SURE program

Title: Effects of medial entorhinal cortex lesions in rats on the Traveling Salesman Problem

Authors: L. OLIVAS[1], D. ABOUCHEDID[1], *R. BLASER[1], J. B. HALES[2];
[2]Psychological Sci., [1]Univ. of San Diego, San Diego, CA

Abstract: Although highly controlled behavioral tasks are valuable for isolating and targeting specific processes, they risk producing too narrow an understanding of behavior in natural contexts. The Traveling Salesperson Problem (TSP) is a spatial navigational task that differs from simple spatial memory tasks; rather than examining an animal's ability to perform a precise behavior, it explores foraging behaviors in a naturalistic setting. While foraging is a spontaneous behavior, it is also complex, in that it involves decision-making, attention, spatial processing and navigation, course planning, and memory. Experiments using simple, highly-controlled conditions have indicated that the medial entorhinal cortex (MEC) plays an important role in spatial processing and spatial memory in ways that are similar to, and yet distinct from, those of the hippocampus. Therefore, comparing the effects of MEC lesions to those of hippocampal lesions on rats' performance in the TSP task may help clarify the relative contributions of these different anatomical brain areas to spatial navigation under more ecologically relevant conditions. Previous research from our lab found that rats with hippocampal lesions were impaired on their performance of the TSP task in comparison to sham controls, particularly on measures of spatial memory, such as making more errors by revisiting targets and making fewer

consecutive retrievals prior to the first revisiting error. In our currently study, we found that MEC lesions also impaired performance on the TSP, although to a more limited degree than hippocampal lesions. Like rats with hippocampal lesions, those with MEC lesions showed more revisit errors and longer travel distances than those with sham lesions. Experimental rats also showed a greater tendency to circle the perimeter of the arena, possibly as a strategy to cope with reduced spatial working memory.

Disclosures: L. Olivas: None. **D. Abouchedid:** None. **R. Blaser:** None. **J.B. Hales:** None.

Poster

P864. Learning, Memory, and Spatial Navigation: Rodent Models

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Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P864.05

Topic: H.09. Spatial Navigation

Title: Neural activity in entorhinal cortex-led processing loops evoked by exposure to a known or new environment

Authors: *C. H. GODFREY, E. M. BUNCICK-ROAKES, K. M. GEISINGER, J. L. REGAN, G. M. GRAVEEL, M. C. ZRULL;
Appalachian State Univ., Boone, NC

Abstract: Perception of the environment happens in less than a moment and permits movement through a space, navigation to or around objects, and reaching a goal location. Using adolescent Long-Evans rats (n=20), we examined two processing loops, one led by superficial medial entorhinal cortex (MEC) thought to process information about the global scene and path navigation and the other led by superficial lateral EC (LEC) that may process information regarding objects and their locations. Structures in each loop were explored using an environmental enrichment paradigm (EE) and by assessing c-FOS expression in neural regions in each loop following exposure to an environment with objects in animals with and without a history in the environment (EE+EE, No+EE groups, respectively). Control groups with and without EE history did not have a final EE experience (EE+No, No+No), and EE+EE and EE+No groups experienced 18 periodic EE sessions through adolescence. Counts of active, c-FOS+, neurons were made with digital microscopy, and data were standardized to No+No counts. Regardless of processing loop or region, there were no differences between control groups. In the MEC-led, “global scene/navigation” processing loop, more neurons were activated in superficial MEC (MEC)and proximal CA1 of No+EE (+200%, +390%, $p<.01$) and both No+EE and EE+EE (+350%, +690%, $p<.01$) than in controls; however, in distal subiculum and deep MEC enhanced activation was similar for No+EE and EE+EE groups (+375%, +140%, $p<.01$). In the LEC-led, “object/object location” processing loop, more neurons were activated in superficial LEC of both No+EE and EE+EE (+60%, $p<.02$) and in distal CA1 of No+EE (+355,

p<.01) and both No+EE and EE+EE (+600%, p<.01) than in controls; however, in proximal subiculum and deep LEC enhanced activation was similar for both No+EE and EE+EE groups (+325%, +55%, p<.01). Differences in evoked activity between EE+EE and No+EE groups in superficial MEC but not in superficial LEC support the idea of MEC processing global scenes and LEC processing local cues with similar activity patterns in both proximal and distal CA1 across EE+EE and No+EE groups suggesting subsequent integration of global and local information into a more complete representation of a space. The data suggest subiculum, in both loops, likely modifies information as well as simply acting as a relay back to deep MEC and LEC. While our results show support for differences and similarities in processing global and local cues though MEC/LEC to hippocampus loops, they also point to the need for further research using various methodologies.

Disclosures: C.H. Godfrey: None. **E.M. Buncick-Roakes:** None. **K.M. Geisinger:** None. **J.L. Regan:** None. **G.M. Graveel:** None. **M.C. Zrull:** None.

Poster

P864. Learning, Memory, and Spatial Navigation: Rodent Models

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P864.06

Topic: H.09. Spatial Navigation

Title: Evoked activity in superficial entorhinal cortex after exposure to an environment with or without objects

Authors: H. B. WOLFE, K. M. GEISINGER, E. A. GIBSON, J. L. REGAN, ***M. C. ZRULL**; Appalachian State Univ., Boone, NC

Abstract: The entorhinal cortex (EC) leads, and ends, two neural processing loops through the hippocampal formation (HF). In part, activity across two loops, one beginning with medial EC (MEC) and the other with lateral EC (LEC), contributes to neural representations of an animal's environment as a whole (e.g., MEC led loop) and the location of items within that environment (e.g., LEC led loop). In this initial study, we used c-FOS to examine neural activation in Layers 2 and 3 of MEC and LEC, which receive input derived from a range of brain regions and relay input to the HF, evoked by exposure to enriched environments (EEs) with or without a variety of moveable objects. Subjects were 15, adolescent Long-Evans hooded rats. After weaning, the animals were housed in groups in standard shoebox cages and handled daily. At 49 days old and prior to sacrifice, 5 rats spent 1.5 h in an enclosure with ramps and platforms (NoObj group), 5 rats spent 1.5 h in an EE but with a number of objects (YesObj group), and 5 rats spent 1.5.h in a standard shoebox cage in a quiet and dark room (control). Brains were processed to visualize c-FOS+ neurons, and cell counts were made using digital microscopy and stereological technique. All data were standardized to the control group cell counts. Enhanced neural activity was

observed across Layers 2 and 3 of MEC and LEC of both NoObj and YesObj groups in comparison to controls (all $p<.01$). For LEC Layer 2, NoObj and YesObj groups did not differ in c-FOS+ neuron counts; however, 147% more activated neurons were observed in LEC Layer 3 of NoObj than YesObj brains ($p<.037$). Both Layer 2 and 3 of MEC in NoObj brains exhibited enhanced evoked activity relative to YesObj brains (+133%, $p<.017$ and +827%, $p<.001$, respectively). These results were somewhat unexpected. MEC layers were expected to be activated similarly across NoObj and YesObj groups due to responsivity to spatial cues of the overall environment (i.e., the global scene). However, more neural activity was elicited in three of four EC layers by exposure to an environment devoid of moveable objects than a setting with various objects (i.e., NoObj vs. YesObj groups). In contrast, and more expectable, more c-FOS+ neurons were found in MEC than LEC (+46%) of the NoObj group, and within the YesObj group more activated neurons were in LEC than in MEC (+71%). These latter results align with suggestions that while MEC responds to the global nature of an environment, the LEC responds to both the space of an environment and the location of various objects within that space. That said, our data seem to suggest the EC may be more responsive to the global nature of an environment than local cues within that space.

Disclosures: H.B. Wolfe: None. **K.M. Geisinger:** None. **E.A. Gibson:** None. **J.L. Regan:** None. **M.C. Zrull:** None.

Poster

P864. Learning, Memory, and Spatial Navigation: Rodent Models

Location: Virtual Only

Time: Thursday, November 11, 2021, 2:30 PM - 3:30 PM

Program #/Poster #: P864.07

Topic: H.04. Executive Functions

Title: Fluoxetine as a novel treatment to attenuate cisplatin-induced cognitive impairment

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Abstract: The American Cancer Society estimates that there are 4,950 new cancer cases each day in the US, and ¾ of that population may suffer cognitive decline because of treatments that were necessary to save their lives. Cisplatin, a chemotherapeutic agent, initiates cell death through various methods that include mtDNA damage during cell replication and produces damaging pro-inflammatory cytokines. Unfortunately, the damage caused by cisplatin treatment is not selective to only cancer cells but also damages healthy cells. This can result in long-term chemotherapy-induced cognitive decline (CICD). Fluoxetine (FLX) is a selective serotonin reuptake inhibitor widely used in patients with anxiety and mood disorders. Published studies report that FLX could sensitize cancer cells to chemotherapy treatment and assist in attenuating

CICD. We hypothesize that the combined therapy of Cisplatin and FLX will increase the sensitivity of cancer cells to Cisplatin and protect against CICD. Methods include both *in vitro* and *in vivo* studies. To assess the ability of FLX to sensitize cancer cells to Cisplatin, we used established cancer cell lines and various doses of FLX or Cisplatin to determine the dose that achieved 50% cell death. Optimal doses were combined, and cytotoxicity was measured. To determine whether FLX treatment could reduce CICD, we used C57 mice and a delayed non-match-to-sample (DNMS) or delayed-match-to-sample (DMS) operant tasks to assess learning and cognitive flexibility. Treatment cohorts included saline/saline, cisplatin/saline, FLX/saline, and FLX/Cisplatin. The treatment regimen included two 5-day cycles of Cisplatin treatment with 5 days rest in between. FLX or saline was administered for the entire 15 days. Mice were then trained to lever press for reinforcement under either DMS or DNMS conditions. Following successful acquisition of the task, reinforcement conditions were reversed. Results indicate that FLX sensitizes cancer cells to cisplatin treatment *in vitro*, and *in vivo* cisplatin induces CICD that FLX is able to attenuate. Combined treatment of FLX and Cisplatin may reduce undesirable side effects by using a lower chemotherapeutic dose to achieve the same rate of cancer cell death, and FLX may help protect healthy cells from chemotherapy-induced toxicity. This research will ultimately provide scientists and medical professionals with new information on why patients may experience alterations in their cognitive function and potentially offer a new treatment option to improve the overall quality of life. Further studies are necessary to explore the underlying neuronal changes that occur with cisplatin treatment and how FLX attenuates CICD.

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