Conference Agenda

MON	TUE	WED	THU
08 June	09 June	10 June	11 June

Program Overview

Date: Monday, 08/June/2026

11:30am - 12:00pm	**Registration** Location: **Foyer at Assembly Room entrance**
12:00pm - 4:00pm	**Trainee Workshop & Luncheon: Networking for Success in Science** Location: **Assembly Room**
4:00pm - 5:30pm	**Data Blitz: Data Blitz** Location: **Assembly Room/Ballroom**
6:00pm - 7:00pm	**Plenary 1: Presidential Address:** Location: **Assembly Room/Ballroom** **From GWAS to Function: finding genetic mechanisms for brain disorders** **Danielle Posthuma** Vrije Universiteit Amsterdam Danielle Posthuma Genome-wide association studies (GWAS) have successfully identified many novel loci for neuropsychiatric traits. At the same time the results of GWAS showed that these traits are highly polygenic, mostly influenced by large numbers of weakly associated variants. Interpreting such polygenic results is challenging. Recent large-scale initiatives, such as those from the Allen Brain Institute and the PyschEncode consortium provide fine-scaled atlases of functional genetic elements at cellular level. This novel information can be used to interpret results from GWAS studies and facilitate biological understanding of complex traits. In this session, I will discuss how we can leverage both GWAS results and novel functional genomic resources to formulate hypotheses that can be tested in functional experiments. I will discuss current and just finished work on gene finding for brain related traits, including strategies to link genetic findings to biology or drug targets. This includes the latest results - hot off the press - from a large GWAS on Alzheimer's disease for the PGC-ALZ working group, and several new findings for psychiatric disorders, including schizophrenia. Vrije Universiteit, Amsterdam, NL
7:00pm - 9:00pm	**Opening Reception** Location: **Lower Lounge**

Date: Tuesday, 09/June/2026

8:00am - 8:30am	**Registration** Location: **Foyer at Assembly Room entrance**
8:30am - 10:00am	**Outstanding Travel Awardee Presentations** Session Chair: **Cheryl Reed** Session Chair: **Karissa Reyes**

Characterization of Dpp6 effects on ethanol consumption, reward, and locomotor behavior

Amanda Barkley-Levenson
University of New Mexico

M Hernández[1], R Sultana[1], D-J Paredes[1], AM Barkley-Levenson[1]

Recent genome wide association studies (GWAS) have identified numerous novel hits for problematic alcohol use and alcohol consumption. However, follow-up studies are still needed to demonstrate a causal relationship between implicated genes and alcohol-related traits. Here, we describe the functional validation of *Dpp6*, which is a novel genetic association for problematic alcohol use and alcohol drinking. *Dpp6* encodes an auxiliary subunit of A-type voltage-gated potassium channels and is involved in modulating dendritic excitability and synaptic plasticity. We have found that global knockout of *Dpp6* does not alter ethanol binge-like drinking or total consumption in a chronic intermittent two-bottle choice procedure, but does produce an escalation in ethanol preference over time in this procedure compared to wild type (WT) littermates. Knockout mice also show greater binge-like sucrose intake in a single bottle procedure, but do not differ from WT in sucrose preference in a two-bottle choice test. However, we do see a significant increase in ethanol sensitivity in the knockout mice compared to WTs across multiple behaviors (ethanol conditioned place preference, locomotor sedation, and ethanol-induced anxiolysis) following ethanol injections, suggesting that route of administration may be relevant for the genotypic differences observed in this model. Taken together, these findings confirm that loss of *Dpp6* does impact multiple ethanol-associated behavioral phenotypes, even without significantly altering voluntary ethanol consumption.

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Funding support: NIH-NIAAA grant R00AA027835, NIH-NIGMS grant K12GM088021

Dopaminergic targets of neonicotinoid action

Karina Piotrowska
University of Oxford

Karina Piotrowska1 , Annie Park1 , Scott Waddell1 1

Nicotinic acetylcholine receptors (nAChRs) are implicated in the reinforcing properties of addictive substances. However, their heterogeneous composition makes it difficult to resolve how cell-type specific subunit combinations contribute towards addictive behaviour in vivo. Neonicotinoid insecticides are known nAChR agonists, and have been implicated in pollinator decline due to their addictive properties. We are studying these addictive behaviours in Drosophila, which permits an analysis of the roles of specific nAChR subunits within the dopaminergic system. Quantitative analysis of feeding revealed that flies form a robust dietary preference for sucrose laced with neonicotinoids. Moreover, chronic neonicotinoid exposure results in further elevated consumption of pesticide-laced sucrose, reflecting an experience-dependent adaptation in reward valuation. Optogenetic inhibition revealed an unexpected role for aversively reinforcing dopaminergic neurons in promoting neonicotinoid preference. Single-cell sequencing data of subtypes of dopaminergic neurons reveals differential cell-type specific expression of nAChR subunits. We are currently investigating whether this potential heterogeneity of nAChR composition drives functionally distinct cellular responses to neonicotinoids in vivo. Our studies will generate a cell-type resolved model of neonicotinoid function within dopaminergic circuits, which should uncover conserved neural mechanisms underlying neonicotinoid, and nicotine, addiction.

Centre for Neural Circuits and Behaviour, Department of Physiology, Anatomy and Genetics, University of Oxford

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Clusterin gene as a modulator of nicotine reward and astrocyte morphology

Myra Bower
University of Colorado Boulder

Myra Bower[1,2], Andrew Lombardi[1], Cate Hensley [2], Curtis Borski[1,2], Kora Kastengren[1,2], Erika Mehrhoff[1,2], Charles Hoeffer[1,2] Marissa Ehringer[1,2], Jerry Stitzel[1,2]

Tobacco use remains the world's leading cause of preventable death and disease. Nicotine use disorder is characterized by immediate neuronal adaptations that may promote use. While studies of neurons are informative, a critical perspective is missing for other cell types. Astrocytes are dynamic regulators of brain homeostasis and active participants of neurocircuitry underlying substance use disorders. Clusterin (CLU), a gene identified by large scale human genome wide association studies (GWAS) of smoking, is a gene also primarily expressed by astrocytes. Using a mouse model, we investigated the role of Clusterin in

nicotine induced astrocyte morphology and reward behavior. Immunohistochemical staining for area and Sholl analysis of tissue collected 24 hours after nicotine or saline control identified differences due to Clusterin knockout. Mouse astrocytes were assessed in both primary cell culture exposed to nicotine (100 um) and in adult hippocampus after subcutaneous nicotine injection (0.35 mg/kg). Clusterin loss reduced the morphological response of astrocytes to nicotine in vitro and in vivo. Clusterin knock-out and wild-type mice were also tested for nicotine reward by conditioned place preference at the same dose. Additionally, we also see a trend ($p = 0.07$) for Clusterin knock-out towards a place aversion to nicotine, suggesting loss of the gene induces a more unpleasant experience at this dose. These data support Clusterin as a genetic modulator of nicotine-conditioned responses and glial plasticity that may prevent nicotine use in mice.

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GWAS of aversion-based learning behaviors in Heterogeneous Stock Rats identifies novel associations for punishment resistance, cocaine avoidance, and locomotor activity

Zachary Tatum
Virginia Commonwealth University

Zachary Tatom1 , Maya Eid2 , Thiago Missfeldt Sanches1 , Apurva S. Chitre1 , Denghui Chen1 , Benjamin Johnson1 , Elaine Keung1 , Oksana Polesskaya1 , Tom Jhou2 , Abraham A. Palmer1,3

Many substances of abuse produce initial rewarding eHects (euphoria) followed by a period of aversive eHects including anxiety, craving, anhedonia, and withdrawal. Dysregulation of these aversive eHects has been suggested to contribute to the etiology of substance use disorders through aberrant avoidance-based learning. We hypothesize that GWAS of avoidance-based learning using both food-based and cocaine-based behavioral assays in Heterogenous Stock (HS) rats will enable estimation of the heritability of these behaviors and identify new loci associated with them in rats. We exposed 1,074 HS rats (379 male, 695 female) to behavioral testing including runway operant cocaine-seeking, food-based progressive ratio and punishment testing, and locomotion assays. Our results demonstrate that behavioral phenotypes derived from these assays are significantly heritable (with h2 estimates ranging from 0-0.307) and identify significant ($p < 0.05$) genetic loci related to avoidance-based learning including from the punishment task on Chromosomes 2, 3, 5, and 6, and the cocaine-operant runway latency task on Chromosome X. 172 positional candidate genes were identified from significant and suggestive loci, including genes from the cadherin family and genes associated with primary neuronal cilia. Lead GWAS SNPs were also associated with novelty-related and social interaction phenotypes in PheWAS from independent samples of HS Rats. Our results suggest that these avoidance-based learning traits are themselves complex heritable traits which may pleiotropically aHect other aspects of addiction biology.

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10:00am - 10:30am — **Break**
Location: **Assembly Room**

10:30am - 12:30pm — **Symposium 1: Encoding and Decoding Behavior: Computer Vision for Genetic and Neural Analysis**
Location: **Assembly Room/Ballroom**
Session Chair: **Jacob Beierle**
Session Chair: **Gregory Corder**

Behavioral State Space Modeling Reveals Hidden Structure in Spontaneous Pain and Analgesia

Gregory Corder
University of Pennsylvania

Genotype-dependent behavioral signatures of opioid withdrawal revealed by automated behavioral quantification

Jacob Beierle
Jackson Laboratory

Mapping the landscape of social behavior

Ugne Klibaite
Harvard University

Decoding neuronal regulation of aggression across sexes using Drosophila

Catherine Schretter
Southern Methodist University

12:30pm - 1:30pm	**Lunch** Location: **Lower Lounge/Ballroom**
1:30pm - 3:30pm	**Symposium 2: From Sequence to Structure: Decoding the Gene Regulatory Grammar of Addiction** Location: **Assembly Room/Ballroom** Session Chair: **Francesca Telese**

Long noncoding RNAs form R-loops to shape emotional experience–induced behavioral adaptation

Makoto Taniguchi
Medical University of South Carolina

Rose Marie Akiki, Sierra Simmerson, Caila Worley, Rebecca G Cornbrooks, Kosuke Magami, Alain Greige, Kristen K Snyder, Daniel J. Wood, Mary Claire Herrington, Philip Mace, Kyle Blidy, Nobuya Koike, Stefao Berto, Christopher W. Cowan, Makoto Taniguchi

Emotional experiences often drive behavioral responses that enable organisms adapt to their environment. These experiences trigger the expression of specific gene, initiating transcriptional programs that underlie cellular, synaptic, and behavioral plasticity. This gene expression is tightly regulated and plays critical role in adaptive responses to emotional stimuli. Conversely, dysregulation of these transcriptional program is implicated in maladaptive behaviors associated with neuropsychiatric disorders, including mood and substance use disorder (SUD). We recently demonstrated that long non-coding RNAs (lncRNAs) form DNA:RNA hybrid structure, R-loops, across the genome. Genes associated with these R-loops are identified in the glutamatergic and dopaminergic signaling pathways, calcium signaling pathways, and synaptic plasticity-related genes. We discovered that neuronal activity induces the formation of R-loop at the enhancer region of immediate early gene, *Npas4*, a gene that we previously identified as a key regulator of stress-induced anhedonia-like behavior and drug reward-associated contextual memory. This R-loop is essential for linking distal enhancer to the proximal promoter, facilitating *Npas4* gene expression, and regulating cocaine reward-associated contextual memory. Together, these data identify R-loops as novel regulatory mechanism that translates emotional experience into gene expression and behavioral plasticity. In this presentation, we will discuss the functional mechanisms of R-loops in gene expression and behavior in response to emotional experiences.

Department of Neuroscience, Medical University of South Carolina

Funding support: NIH_T32 DA07288, R01 DA032708, R01 MH129521, P20 GM140964, P20 GM148302, P50 DA046373, and UL1 TR001450.

Nucleus accumbens Drd3 medium spiny neuron abundance is associated with opioid in-take in outbred rats

Francesca Telese
University of California, San Diego

Brad Balderson1 , Narayan Pokhrel2 , Arnav Gurha2 , Yanning Zuo2 , Benjamin Johnson2 , Olivier George2 , Abraham A. Palmer2,3 , Graham McVicker1 , Francesca Telese2

The nucleus accumbens (NAc) is a key subcortical brain structure that regulates reward and is involved in addiction. However, the molecular basis of individual differences in addiction phenotypes are not well characterized. To dissect the molecular basis of oxycodone addiction, we used an intravenous self administration assay (IVSA) to measure oxycodone addiction-like behaviours in outbred Heterogeneous Stock (HS) rats. NAc tissues were collected from 85 HS rats after 5 weeks of abstinence from oxycodone. We obtained whole genome sequencing, and also 10X multi-ome from 500,000 single-cell nuclei. Latent factor analysis on gene expression and chromatin accessibility across 14 NAc cell types identified a molecular pattern of changes significantly associated with oxycodone in-take (factor of oxycodone, FOXY). A major driver of FOXY was the abundance of Drd3-expressing medium spiny neurons (MSNs), with higher abundance associated with increased oxycodone in-take. We also identified gene expression and chromatin accessibility changes in Grm8-expressing MSNs and Chat expressing interneurons as a major component of FOXY. These cell types exclusively express the opioid receptor Oprm1 in the NAc. To evaluate if FOXY was genetically driven, we called cis-acting eQTLs and caQTLs across all cell types, and trained Predixscan models to predict gene expression and chromatin accessibility for identified eGenes and caPeaks in each cell type. These cis-variant predicted expressions indicated 18% of the variance in FOXY could be explained by genetic differences between individuals, and this variance was marginally predictive of oxycodone-intake (R=0.18, p=0.14). Overall, we identified a pattern of molecular and cell abundance changes in the NAc associated with oxycodone in-take, and at least 18% of the variance in this pattern was explained by genetic differences between individuals.

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A single cocaine exposure rewires the 3D genome structure of midbrain dopamine neurons

Ana Pombo
Johns Hopkins

Ana Pombo

Midbrain dopamine neurons (DNs) are the first responders to the acute exposure of cocaine and are central to the development of drug addiction. Following a single cocaine injection, DNs undergo synaptic and transcriptional changes that are resolved within days but preserve a long-term cellular memory of the exposure by a mechanism that remains unknown. To investigate whether persistent alterations in 3D genome organization are involved in the cellular memory of an acute drug exposure, we applied Genome Architecture Mapping (GAM), single nucleus transcriptomics (snRNA-seq) and chromatin accessibility mapping (snATAC-seq) in the mouse midbrain 24 hours or 14 days after a single injection of cocaine or saline control. We found that DNs retain extensive chromatin reorganization 14 days after a single exposure across multiple genomic scales, including topologically associating domains (TADs), chromatin condensation and looping interactions, which affect genes previously associated with chronic cocaine addiction, synaptic plasticity and metabolic adaptation.To assess whether altered 3D genome structure 14 days post exposure has consequences for the transcriptional responses to cocaine, we re-exposed animals to a second injection of cocaine after 14 days of abstinence. Transcriptional responses were more extensive and often did not match the direction or intensity of changes seen upon the first exposure. Importantly, transcriptional responses following re-injection occurred predominantly at genes with reorganized chromatin architecture. Together, these findings identify 3D genome remodelling as a key mechanism underlying cellular memory of cocaine exposure, providing new insight into the earliest molecular events in addiction and the plasticity of the genome.

Max Delbrück Center for Molecular Medicine, Berlin Institute for Medical Systems Biology, Epigenetic Regulation and Chromatin Architecture Group, Berlin, Germany.

Department of Biology and Department of Molecular Biology and Genetics, Johns Hopkins University, Baltimore, MD, USA.

Re-engineering brain transcription factors connects transposable elements and neuroimmune response to cocaine-use

Peter Hamilton
Virginia Commonwealth University School of Medicine

Gabriella M. Silva[1], Joseph A. Picone[1], Annalise Hassan[1], Natalie L. Truby[1], R. Kijoon Kim[1], Corinne Smith[1], Shelbey S. Strandberg[1], Jessica L. Bell[1], Xiaohong Cui[1], Theingi Aung[1], **Peter J. Hamilton[1]**

Zfp189, which encodes a Krüppel-associated box zinc finger protein (KZFP) transcription factor (TF), differentially accumulates in rodent cortical and limbic brain regions in response to stress- or drug-use experience. Here, we aimed to illuminate the brain cell-type specific molecular mechanisms through which this and other KZFP TFs produce cocaine-related brain changes, with emphasis on investigating transposable elements (TEs), since KZFPs like ZFP189 are known regulators of TEs. To investigate this, we quantified TE transcripts in existing single nuclei and bulk RNA-sequencing datasets of rodents exposed to cocaine. TE transcripts often go un-analyzed in transcriptomic data due to multi-mapping reads. We identified dynamic TE transcript expression across cocaine exposure, especially in nucleus accumbens (NAc) medium spiny neuron (MSN) subtypes. To directly regulate brain TEs, we created novel synthetic ZFP189 TFs and synthetic KZFP-interacting TRIM28, each capable of exerting distinct forms of transcriptional control at KZFP-regulated genes, including TEs. These KZFP TFs were virally delivered to the NAc of mice, including conditional delivery to MSNs, and the consequences on cocaine-related behaviors, including intravenous self-administration, and transcriptional response, by bulk and snRNAseq, were characterized. We discover that normal KZFP function in brain is critical to produce cocaine-induced NAc transcription and an escalation of cocaine-taking behaviors, and this can be impeded with synthetic KZFP TFs. Our synthetic KZFPs release TEs that form cis-regulatory contacts with down-regulated, predominantly immune-related genes. Collectively this work points to the KZFP-mediated transcriptional repression of brain TEs as an important mechanistic step in cocaine-induced gene expression and behavioral changes.

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Funding from NIH grants: R01DA058089, R01DA058958

3:30pm - 4:00pm

Break
Location: **Assembly Room**

4:00pm - 5:30pm

Plenary 2: Keynote Address (Professor Erich Jarvis)
Location: **Assembly Room/Ballroom**
Session Chair: **Sean Farris**
Session Chair: **Daniela Gil**

Brain pathways for vocal learning and spoken language

Erich Jarvis
Howard Hughes Medical Institute, Rockerfeller University

5:30pm - 7:00pm

Posters A: Poster Session A
Location: **Assembly Room/Kurtzman Room**

Poster 2: Behavioral and Transcriptome Effects of a Trace Amine-Associated Receptor 1 Null Mutation on an Isogenic C57BL/6J Genetic Background

Cheryl Reed
Oregon Health & Science University

Cheryl Reed[1], Grant Moen[1], Sahana Srinivasan[1], Shauna Rakshe[2], Suzanne Fei[2], Jason Erk[1], Tamara Phillips[1,3]

Selective breeding for methamphetamine (MA) intake identified a null mutation in the trace amine-associated receptor 1 (*Taar1*) gene that negates TAAR1 function and increases genetic risk for MA intake in mice. A knock-in (KI) was produced on an isogenic C57BL/6J background, replacing the reference *Taar1*$^{+}$ allele with the mutant *Taar1*^{m1J} allele. Increased MA intake in *Taar1*^{m1J} mice was verified. Here, we examined the effects of *Taar1*^{m1J} on MA-induced locomotor stimulation and conditioned taste aversion (n=15-16 and 6-9 mice/genotype/sex/dose, respectively) and characterized the transcriptome using RNA-sequencing data from MA-naïve mice (n=12 mice/genotype/sex) for the nucleus accumbens, prefrontal cortex, and ventral midbrain (VMB). The allele replacement did not alter MA-induced locomotor stimulation but attenuated MA-induced conditioned taste aversion (2 and 4 mg/kg MA; ps<0.001). Transcriptome analysis identified 1,326 differentially expressed genes (p<0.05), with 56 shared across brain regions. QIAGEN Ingenuity Pathway Analysis identified 265 enriched pathways, three of which included *Taar1* (NF1 RAS Signaling Pathway, Phagosome formation, and Cellular Effects of Sildenafil). Weighted gene co-expression network analysis identified modules that differed (ps < 0.05) between *Taar1* genotypes only in the VMB. Enriched gene ontology processes included: neuron ensheathment, myelination, glial cells, synaptic transmission, synaptic assembly, synaptic regulation, and neural development/regulation. Nineteen hub genes were identified including: *Litaf*, *Trp53inp2*, *Insc*, *Gltp*, *Tmprss5*, *Gpr37, Car14, Ids*, *Lonrf2*, *Camsap2, Dzank1, Ppp1r9a, Arhgap20, Cmtm4, Sox10*, *Fa2h*, *Plekhg3*, *Tmem63a*, and *Jup*. The underlined hub genes were previously found to be involved in MA-related behavioral and molecular changes. These processes may underlie TAAR1-mediated MA aversion impacting MA intake.

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Poster 4: Regulation of binge ethanol consumption and prefrontal cortex glutamate neurotransmission and glutamate transporter expression by STAT3 in astrocytes

Amy Lasek

Virginia Commonwealth University

AW Lasek1, M Galan-Llario1, H Chen1, E Legge1, J Almeida1, L Carvalho1, CM Erikson2, R Vlkolinsky2, M Bajo2, and M Roberto2

STAT3 is a transcription factor activated downstream of cytokine, chemokine, and growth factor receptor stimulation. It plays an important role in the innate immune response by promoting astrocyte reactivity and neuroinflammation in response to tissue injury. STAT3 is activated in several brain regions following chronic ethanol exposure in mice, rats and humans. To determine if astrocyte-expressed STAT3 regulates ethanol consumption, we generated conditional astrocyte *Stat3* knockout (Stat3aKO) mice. Male, but not female, Stat3aKO consumed less ethanol than controls. To determine potential mechanisms contributing to decreased ethanol consumption in the Stat3aKO mice, we recorded glutamate receptor-mediated spontaneous excitatory post-synaptic currents (sEPSCs) in prelimbic pyramidal neurons in male Stat3aKO and control mice. We found a significant increase in the mean baseline sEPSC amplitude, but not frequency, in Stat3aKO mice. As astrocytes are involved in uptake of synaptic glutamate, we hypothesized that STAT3 might regulate the expression of glutamate transporters and measured transcript levels of *Slc1a2*, *Slc1a3*, *Slc7a11*, and *Slc17a8* in Stat3aKO and control mice. *Slc1a2* and *Slc17a8* were significantly decreased in both male and female Stat3aKO mice, although the magnitude of the decrease was greater in males. These results suggest impaired glutamate clearance in male Stat3aKO mice, and that STAT3 in astrocytes promotes binge drinking. In conclusion, this study links neuroimmune signaling in astrocytes to cortical excitatory neurotransmission and ethanol consumption.

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Poster 6: Studying the role of Orb2 in encoding drive buildup in NPF neurons

Michaela Meshi

Bar Ilan University

Meshi Micheala[1], Levi Mali[1] & Shohat-Ophir Galit[1]

Motivation is an internal representation of physiological needs that drives goal-directed behavior, ensuring that actions are expressed at the appropriate time, intensity, and context. Although the neurobiology of motivation has been extensively studied, the mechanisms by which motivational drives accumulate over time and are translated into sustained behavioral states remain poorly understood. Konrad Lorenz's hydraulic model proposes that motivation builds gradually like pressure in a reservoir, yet the existence and nature of a corresponding biochemical mechanism remain unclear. In this study, we tested the hypothesis that drive buildup is encoded by the accumulation of prion-like proteins within neurons that function as motivational hubs. We focused on the Drosophila neuropeptide F (NPF) system, which is

homologous to the mammalian NPY system, and examined the role of the prion-like RNA-binding protein Orb2, which undergoes state-dependent oligomerization and regulates local protein synthesis at synapses. Using microscale thermophoresis, western blotting, and confocal imaging, we quantified Orb2 monomeric and oligomeric states in vivo under distinct internal drives. The result demonstrate that prolonged starvation, but not acute water deprivation, induced increased Orb2 accumulation and a shift toward oligomerization in NPF neurons, with enrichment at synapses of a subset of neurons projecting to the fan-shaped body and suboesophageal zone. This accumulation was reversible upon refeeding. In contrast, neurons expressing the NPF receptor showed reduced Orb2 accumulation during food deprivation, consistent with reciprocal signaling. Functionally, Orb2 oligomerization was required for starvation-induced synaptic localization of neuropeptide F released from NPF neurons. Together, these findings support a model in which Orb2 acts as a reversible molecular integrator of metabolic state, linking internal energy deficit to sustained neuromodulatory remodeling in feeding-related circuits, and providing a framework for future work on graded drive and downstream RNA targets.

1 The Mina & Everard Goodman Faculty of Life Sciences, The Multidisciplinary Brain Research Center and, Institute
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Poster 8: Systems Genetics of Fentanyl Addiction in the Collaborative Cross

Nathan Bolen
Marshall University

Nathan Bolen, Colton J. Treadway, Michael Leonardo, Wes Tackett, Aneesh Gupta, Joshua Sisco, Travis B. Salisbury, James Denvir, Sadia Akter, Vini Magalhaes Borges, Alejandro Q. Nato, Jr., Brandon J. Henderson, Price E. Dickson

In 2024, 47,735 people in the United States died from synthetic opioid overdose, primarily due to fentanyl (CDC, 2026). This striking statistic underscores the urgent need to discover and characterize the genetic underpinnings of fentanyl addiction. To this end, we are using genetically diverse mouse strains (N = 40) from the Collaborative Cross (CC) and their founders to discover genetic, transcriptomic, epigenomic, and neurophysiological mechanisms underlying fentanyl addiction-like behaviors and addiction endophenotypes. Notably, the CC founder strains encompass ~90% of known genetic diversity in laboratory mice originating from *Mus musculus*. We are testing mice on four behavioral assays: intravenous fentanyl self-administration, sign-tracking/goal-tracking, fentanyl locomotor sensitization, and operant sensation seeking. Machine learning enables nuanced analysis of these mouse behaviors. We are collecting nucleus accumbens punches following sign-tracking/goal-tracking and fentanyl locomotor sensitization; gene expression using RNA-seq will be quantified from this tissue. We are using fast-scan cyclic voltammetry, patch-clamp electrophysiology, spatial transcriptomics, single-cell multiomics, and long-read sequencing to characterize CC founder strains, extreme CC strains, or both. Data collection for this study will occur over five years. Here, we report results after ~20 months of phenotyping. Briefly, interim results reveal significant strain effects, GxE effects, and sex effects on many phenotypes. Over the next several years, we will integrate QTL mapping, eQTL mapping, and genetic correlations spanning multiple biological levels. Collectively, these data will provide a foundation for future deep characterization of identified mechanisms, a lasting community resource, and will ultimately contribute to the development of novel, more effective addiction treatments.

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Poster 10: A Systems-Level Map of THC-Induced Brain Signaling Reveals Divergent Genetic and Sex-Dependent Pathways

Aijun Zhang
University of Tennessee, Health Science Center

Aijun Zhang1,2, Lukmon Raji2, Zhiping Wu4, Sufiya Khanam1, Dehui Kong1,2, Ling Li2, Junmin Peng4, Bob Moore3, Xusheng Wang1,2, Megan Mulligan2

Cannabis is the most used federally illegal drug in the United States. Over the past years, cannabis usage has increased among adults. Growing research shows that high levels of ∆9-tetrahydrocannabinol (THC), the major psychoactive component of cannabis, impairs memory, learning, attention, cognitive performance, motivation, and is also associated with brain alterations. Accordingly, there is increasing demand to define targets of cannabinoids and to understand how individual genetic differences moderate response to THC. To address this gap, we developed a holistic approach to quantify the effects of acute THC on brain signaling by integrating multi-omics data collected from genetically divergent C57BL/6J and DBA/2J mice. Both sexes (n=4) were injected with 10 mg/kg THC or vehicle (i.p). Cortex was collected 60 mins post-injection. RNA and protein were extracted from each hemisphere, followed by RNA-seq, proteomics, and phosphoproteomics profiling. The results show that the DBA/2J strain exhibits a larger acute response to acute high dose THC than C57BL/6J, with most differences observed at the phosphosite level. Moreover, multi-omics analysis indicates that the cortical phosphoproteome responds robustly to THC at 60 mins, while whole proteome and transcriptome are less responsive. This is consistent with expectations that phosphorylation acts as an early signaling event preceding downstream transcriptional remodeling and changes in protein abundance. Our preclinical study reveals new molecular targets and signaling pathways underlying individual differences in acute responses to THC that may provide insight into genetic differences in adverse health risks in humans.

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Poster 12: Multidimensional phenotyping reveals strain-dependent variability in opioid-induced mechanical allodynia following oxycodone self-administration

Alanna Mayberry
University of Colorado Boulder

Alanna Mayberry1,2, Eamonn Duffy1,3,4,7, Johnson Ajanaku1,3,4, Laura Saba6,6, Ryan Bachtell2,3,4, Marissa Ehringer1,3,4

Chronic opioid exposure can paradoxically enhance pain sensitivity, expressed behaviorally as opioidinduced mechanical allodynia. This effect is thought to reflect maladaptive nociceptive plasticity, yet the genetic and biological determinants of vulnerability remain incompletely defined. We examined strain- and sex-dependent variation in oxycodone-induced mechanical allodynia, mass and its trajectory, and voluntary opioid intake using a genetically diverse rat panel and applied multivariate analyses to resolve latent dimensions of opioid response. Adult male and female rats (n = 410) from 15 strains of the Hybrid Rat Diversity Panel underwent intravenous oxycodone or saline self-administration. Mechanical withdrawal thresholds (von Frey testing) and body mass were assessed before and after oxycodone exposure. Univariate analyses, principal component analysis, unsupervised k-means clustering, and broad-sense heritability estimation were performed. Oxycodone self-administration produced robust mechanical allodynia and disrupted normal weight gain. Both effects were strongly strain-dependent, whereas sex significantly moderated change in body weight but not allodynia magnitude. Principal component analysis identified four orthogonal dimensions explaining 94% of total variance, dissociating baseline mechanical sensitivity from opioid-induced plasticity and global physiological state. Unsupervised clustering revealed four biologically coherent subtypes distinguished by opioid intake, severity of mechanical hypersensitivity, and body mass. Cluster membership was non-randomly distributed across strain, sex, and treatment condition. Baseline traits demonstrated moderate-to-high heritability, while opioid-induced changes showed reduced heritability, consistent with strong environmental modulation during drug exposure. Oxycodone induces genetically constrained yet heterogeneous neurobehavioral adaptations. Multivariate phenotyping reveals distinct biological routes to opioid vulnerability, underscoring the importance of systems-level approaches in addiction and pain research.

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Poster 14: Adolescent Social Instability Stress Alters Anxiety and Nicotine Sensitivity in Two C57BL/6 Substrains

Carlos Novoa
Pennsylvania State University

Carlos Novoa 1, Thomas J. Gould 1 1 Department of Biobehavioral Health

Adolescence is a sensitive developmental window characterized by profound changes in brain circuitry, amplifying both adaptability and vulnerability to environmental influences. Social stress during this period is a prominent risk factor for adverse psychiatric outcomes later in life. This study investigates the interplay between adolescent social stress and genetic background in determining anxiety-related behaviors and nicotine sensitivity. Using male C57BL/6J and C57BL/6NJ mice, we assessed the physiological and behavioral impacts of adolescent social instability stress (SIS). Our results demonstrate that adolescent SIS significantly impairs body weight gain throughout late adolescence. Regarding behavioral indicators, SIS increases spontaneous locomotion and anxiety-like behaviors in the elevated plus maze. Furthermore, physiological assessments revealed that SIS induces the accumulation of hair corticosterone (CORT) while the HPA response to an acute pharmacological challenge with nicotine was attenuated. Adolescent stress altered subsequent nicotine sensitivity, resulting in a reduced recovery from acute nicotine hypolocomotor effects. Finally, genetic background significantly influenced baseline behavior and drug reactivity, with distinct straindependent differences observed in both anxiety and locomotor activity. These findings underscore the critical role of genetic factors in shaping individual vulnerability to mental health challenges and substance use following adolescent stress.

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Poster 16: Sex and strain differences in the behavioral and brain transcriptional response to repeated delta-9 tetrahydrocannabinol (THC) exposure.

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University of Tennessee Health Science Center

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We compared behavioral and cortical transcriptional responses between sexes and genotypes to identify shared and unique pathways that play a role in tolerance to repeated THC (10 mg/kg, *i.p.*). C57BL/6J and DBA/2J mice of both sexes (*n*=4) were assigned to THC or vehicle (VEH). Following injection of VEH on day 0, mice received four consecutive daily injections of THC or VEH. THC initially induces antinociception, hypomobility, and hypothermia and these traits were quantified daily to track tolerance. On

the 5th day, cortex was collected and RNA extracted for RNAseq analysis. DSeq2 and GSEA were used to identify differentially expressed genes (DEGs) within groups due to treatment and to identify enriched pathways.

Behavioral trait PCA revealed weaker contributions of treatment-associated PCs over exposure days. More treatment DEGs ($p<0.05$) were detected in C57BL/6J (1,291) and DBA/2J (786) females relative to C57BL/6J (216) and DBA/2J (517) males. There was little overlap between groups. Comparison of top enriched genes and pathways between groups revealed shared and unique responses. Ribosome and protein translation was upregulated in C57BL/6J and male DBA/2J but downregulated in female DBA/2J. Mitochondrial metabolic processes were upregulated in C57BL/6 and downregulated in female DBA/2J. Cellular components of neurons were downregulated in C57BL/6. ERK, GTPase, glucocorticoid receptor, and TNFA-NFKB signaling was upregulated in females or DBA/2J females and downregulated in males or C57BL/6 males. Vascular processes were downregulated in males. Identification of individual and shared mechanisms underlying drug tolerance is important for treatment efficacy and understanding the progression towards problem use and addiction.

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Poster 18: Brain transcriptomic profiles of risk for binge-like drinking and selected phenotype preservation for female and male inbred High Drinking in the Dark mice lines

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Alcohol use disorders (AUDs) are known to be polygenetic diseases with high (~0.8) heritability for risk, suggesting that dysregulation of gene expression preceding alcohol exposure may underlie risk. Moreover, there may exist multiple and distinct patterns of gene dysregulation that lead to similar risk for AUDs, thus necessitating the study of multiple, distinct models. We directionally selected the High Drinking in the Dark-1 (HDID-1; 44 generations) and a separate, replicate line (HDID-2; 37 generations) for high blood alcohol following a period of binge-like alcohol drinking from the same genetically heterogeneous progenitor stock (HS/Npt). We report the successful inbreeding of both lines, each starting from their 26th respective selected generation, and the preservation of the selected phenotype (n = 10/sex/fluid/strain; 280 mice). The inbred HDID (iHDID-1&2) lines therefore represent two independently derived, genetically stable models for binge-like alcohol drinking. The two replicate inbred lines are also found to be as transcriptionally distinct as they are similar, relative to their founders. We present differential expression results from alcohol naive mice from both inbred strains and their founders. RNA TAG-seq data were analyzed from six brain regions important for addiction: nucleus accumbens, central nucleus of the amygdala, ventral tegmental area, bed nucleus of the stria terminalis, medial prefrontal cortex, and basolateral amygdala (n = 20/sex/strain/region; 120 mice, 720 samples). We discuss common and unique genetic signatures that are consistent across all six brain regions and their upstream regulators as the most likely targets for therapeutic intervention.

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Poster 20: Characterizing the Impact of Astrocyte Molecular Rhythms in the Nucleus Accumbens on Alcohol Drinking and Reward, Social, and Locomotor Behaviors

Tori Keefauver

University of Pittsburgh

Tori Keefauver[1,2], Nicole L. Horan[1], Lillian Morgan[1], Anisha Saxena[1], Ryan W. Logan[3,4], Gregg E. Homanics[2], Marianne L. Seney[1], Sean P. Farris[2], Kyle D. Ketchesin[1]

Alcohol misuse is a leading cause of preventable death worldwide. Chronic alcohol is associated with disrupted circadian rhythms, yet molecular mechanisms linking circadian rhythm dysregulation and alcohol consumption are poorly understood. Current FDA-approved treatments for alcohol use disorder (AUD) do not target molecular rhythms or sleep-wake cycles. Mammalian circadian rhythms are regulated by transcription-translation feedback loops that regulate the expression of 'clock genes' (e.g. *Clock, Per, Arntl* encoding for BMAL1). Both human and rodent studies have shown clock gene variants are associated with significant changes in reward-seeking behavior. Evidence suggests astrocytes, non-neuronal brain cells with cell-autonomous rhythms, may regulate both circadian rhythms and reward. In the nucleus accumbens (NAc), a region responsible for modulating alcohol- and reward-related behavior, over 43% of the astrocyte transcriptome is expressed rhythmically. No studies to date have investigated the role of NAc astrocyte rhythmicity in regulating alcohol drinking. We used AAV8-Gfap-Cre to functionally ablate molecular rhythms in NAc astrocytes of BMAL1 floxed mice (n = 6/group/sex). Continuous two-bottle choice (2BC), every other day 2BC, and drinking in the dark assessed alcohol drinking. Sucrose preference, social interaction, and light/dark locomotor boxes assessed reward, social, and locomotor

behaviors. BMAL1 functional ablation was not associated with changes in any drinking or behavioral paradigm. Future studies will use operant alcohol self-administration to investigate the role of astrocyte molecular rhythms in motivated alcohol-seeking behavior. Understanding bidirectional relationships between astrocyte molecular rhythms and alcohol consumption will elucidate novel mechanisms of diurnal rhythmicity and inform development of targeted circadian therapeutics for AUD.

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Poster 22: Viral-mediated knockdown of muscarinic M4 receptors exacerbates anxiety- and ethanol-related behaviors in male and female mice

Amine Bahi

Ajman University

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Anxiety disorders frequently co-occur with alcohol use disorder (AUD), and mesolimbic cholinergic signaling has been implicated in the regulation of affective and reward-related behaviors. The muscarinic M4 receptor (M4R) is highly expressed in striatal circuitry, including the nucleus accumbens (NAc), where it may function as an inhibitory modulator of behavioral output. However, its role within the NAc in regulating anxiety-like and ethanol-related behaviors remains unclear. Adult mice received bilateral intra-accumbal injections of viral vectors expressing shRNA targeting M4R or a control construct. Anxiety-like behavior was assessed using the elevated plus maze (EPM) and open field (OF) tests. Voluntary ethanol intake and preference were subsequently measured using a two-bottle choice paradigm. Endpoint M4 mRNA expression within the NAc was quantified using real-time PCR, and expression levels were correlated with behavioral parameters. M4R loss-of-function in the NAc significantly increased anxiety-like behavior, indicated by reduced open-arm exploration in the EPM and decreased center time in the OF test. Following anxiety assessment, accumbal M4-deficient mice exhibited elevated voluntary ethanol intake and preference compared to controls. Saccharin and quinine consumption were unchanged, suggesting enhanced ethanol drinking was not attributable to altered taste sensitivity. Importantly, the degree of M4R mRNA reduction correlated positively with anxiety-like measures and ethanol intake. These findings demonstrate that M4R in the NAc serve as a critical inhibitory regulator of anxiety-like behavior and ethanol consumption, identifying accumbal M4R signaling as a potential therapeutic target for comorbid anxiety disorders and AUD.

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Poster 24: Ethanol preference and intake is reduced by a cross-species healthy fecal microbiome transplantation in female, but not male mice

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Virginia Commonwealth University

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Excessive alcohol consumption decreases gut microbiota diversity and is associated with microbial dysbiosis, defects in the intestinal mucosal barrier, and changes in immune responses. In a germ-free mouse model, our prior work has shown that the beneficial effects of a fecal microbiome transplant (FMT) from treated patients can be transmitted into mice to reduce ethanol intake. Importantly, live microbiota associated with butyrate production, but not their germ-free supernatants, were necessary to reduce drinking. These findings suggest that altering the microbiome in favor of short-chain fatty acid-producing bacteria may be a promising avenue for alleviating the symptoms associated with alcoholic liver disease and drinking. The goal of the current study is to determine what characteristics of FMT donors (bacterial diversity, engraftment efficiency) correlate with the greatest reduction in ethanol consumption. The endogenous gut microbiome was depleted in male and female C57BL/6 mice, followed by administration of one of three healthy human FMT samples and ethanol intake and preference was assessed. We found a robust sex difference in response to cross-species FMT. Two of the three donors significantly reduced ethanol intake and preference in female, but not male, mice. Ongoing metagenomic studies are evaluating microbiota chages during the course of engraftment and drinking to determine the specific characteristics of healthy FMT samples which may most contribute to lasting engraftment and reductions in ethanol drinking. These models of immunocompetent mice with cross-species FMT will provide a foundation for mechanistic studies on gut microbiome modulation as a way to influence ethanol consumption.

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Poster 26: SqueakPose Studio: An end-to-end platform for pose estimation and real-time edge-AI deployment

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National Institute of Health

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Accurate pose estimation underpins quantitative analysis of behavior, yet many deep learning-based tracking tools remain optimized for offline workflows that rely on fragmented software pipelines, workstation-grade GPUs, or external middleware for real-time deployment. Here, we present an integrated software-hardware ecosystem for pose estimation that spans dataset creation, model training, offline analysis, and real-time deployment on embedded edge-computing devices. SqueakPose Studio provides a unified software suite for whole-frame, deep learning-based pose estimation that integrates dataset creation, manual and model-assisted labeling, model training, validation, and large-scale offline inference. For experiments requiring continuous recording and synchronized data acquisition, SqueakView enables real-time model deployment, video capture, and sensor logging on embedded hardware. In parallel, MouseHouse provides a compact, modular enclosure for home cage-based experiments that integrates embedded GPU compute, microcontroller-based timing, and peripheral I/O. A shared data format and deterministic timing architecture ensure consistency between offline analysis and real-time experimentation. Together, SqueakPose Studio, SqueakView, and MouseHouse provide a unified and scalable platform for pose estimation and embedded behavioral experimentation without reliance on workstation-grade hardware or external middleware. This framework enables discovery of previously unobserved behavioral motifs in real time and supports in silico modeling of behavioral sequences relevant to health and disease states.

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Poster 28: A Drosophila model for nicotine reward

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The last 20 years of *Drosophila* addiction research has revealed remarkable similarities between flies and mammals in alcohol and drug-induced behavior and the neural and molecular mechanisms underlying these behaviors. *Drosophila* show parallels to mammals in nicotine-induced behavior and the neural and molecular mechanisms underlying this. Low doses of volatilized nicotine induce an acute startle and hyperactivity responses whereas larger doses decrease locomotion in adult *Drosophila*. Nicotine consumption during development increases heartrate, reduces survival to adulthood, and increases brain size. Like in mammals, nicotine induces dopamine release during both larval and adult stages, and nicotine-dependent changes in locomotion are dopamine dependent. However, there is a gap in our knowledge about whether nicotine is rewarding, and what the genetic and epigenetic mechanisms underlying this response might be. We developed a new conditioned odor preference assay to test the reward responses to nicotine in *Drosophila*, and found that flies reveal appetitive behavioral dynamics favoring an odor previously paired with doses of nicotine that induce acute locomotor hyperactivity. Furthermore, we conducted multiomic analysis of the *Drosophila* mushroom body, a structure required for memory formation, and determined complexity in expression of multiple nicotinic acetylcholine receptors, suggesting that nicotine has strong synergistic effects on cue-induced olfactory memory. Together, our data suggest *Drosophila* is a strong model to test the mechanisms underlying the addictive properties of nicotine.

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Poster 30: Characterizing grimace behavior and microglia morphology in a mouse model of chronic alcohol withdrawal-induced pain

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JD Wherry[1], MR Ross[4], DV Gil[4], GE Homanics[1,2,4], SP Farris[1,3,4]

Alcohol Use Disorder (AUD) is a highly prevalent disease that causes deterioration of health and a steep socioeconomic cost. Individuals with AUD often report symptoms of chronic pain and many continue consuming alcohol to combat these symptoms, especially during withdrawal. Alcohol has deleterious effects on the neuroimmune system that cause aberrant inflammation and increased sensitivity to painful stimuli. However, the neurobiological connection between AUD and chronic pain remains ill-defined. Microglia are the primary immune cells in the central nervous system and mediate neuroinflammation associated with alcohol consumption and pain responses. We hypothesized that exposure to chronic intermittent ethanol vapor (CIEV) would lead to heightened spontaneous nociception (*i.e*. grimacing) and changes in microglial morphology indicative of a reactive phenotype. After five weeks of CIEV, PainFace software showed increased grimacing behavior in mice 24 hours into withdrawal compared to air controls, representative of chronic alcohol withdrawal-induced pain (CAWIP). Grimacing behavior was negatively correlated with territory occupied by microglia in the prefrontal cortex (PFC) indicative of increased microglial reactivity. As a positive control, a separate cohort of mice were injected with lipopolysaccharide (LPS; 1.0 mg/kg) to induce innate immune activation, and grimacing behavior was measured 24 hours post-injection. LPS treatment led to increased grimacing behavior and reductions in territory occupied by microglia compared to saline treatment; similar to mice undergoing withdrawal from CIEV. These data indicate an association between spontaneous nociception and microglial reactivity in mice undergoing withdrawal from chronic ethanol exposure and identify microglia as a possible therapeutic target for treating CAWIP.

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Poster 32: Parabrachial Nucleus Neurons are Sensitized to Mechanical Stimuli during Alcohol Withdrawal

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Alcohol Use Disorder (AUD) is characterized as a chronic medical condition known to be comorbid with pain. Mice experience mechanical and spontaneous pain during alcohol withdrawal, known as chronic alcohol withdrawal-induced pain (CAWIP). We now wanted to characterize a central, brain mechanism that underlies alcohol-induced hypersensitivity during alcohol withdrawal. We hypothesized that the parabrachial nucleus (PBN), a hub for pain and aversion, mediated pain-like responses during alcohol withdrawal. B6J mice were exposed to four weeks of chronic intermittent ethanol vapor (CIEV) or Air (16 hrs ON/8 hrs OFF 4 days/week) to induce pain. During withdrawal, mice underwent 1) immediate early gene (Fos) activation or 2) *in vivo* fiber photometry experiments to measure PBN neuron activity. To induce Fos expression, mice were anesthetized and their left hind paw was stimulated with either a light brush or a noxious pinch. After 90 minutes, brain tissue was taken for histology. For fiber photometry, stimuli, ranging from innocuous to noxious, were applied to the left hind paw of the mice while calcium activity was recorded in real-time. Both PBN Fos expression and PBN calcium activity were increased by non-painful and painful stimulation in mice treated with CIEV when compared to Air controls. Our studies indicate that PBN neurons are more excitable by innocuous (allodynia) and painful (hyperalgesia) stimuli during alcohol withdrawal. Alcohol-induced sensitization of PBN neurons may underlie the hypersensitivity phenotype we see in our laboratory mice during withdrawal from CIEV. Future studies will focus on PBN neuron inhibition to reduce pain.

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Poster 34: Slc39a8 deficiency and ethanol: effects on locomotion, anxiety-like behaviors, and reward sensitivity in mice

André Lucas S. Borges

University of New Mexico

André Lucas Silva Borges, Rebeka Sultana, Amanda M Barkley-Levenson

Alcohol use disorders are known to be major health issues that result in a high number of direct and indirect deaths every year. The gene solute carrier 39a8 (*Slc39a8*), which encodes a transporter of zinc and other metals, has been found in multiple genome-wide association studies (GWAS) to be associated with alcohol consumption and problematic alcohol use. Despite this association, very few studies have characterized the relationship between *Slc39a8* and ethanol-related traits. Thus, we employed ethanol conditioned place preference (CPP) and aversion (CPA), and open field tests to further understand the role of this gene in sensitivity to ethanol's motivational, locomotor, and anxiolytic effects. Adult naïve *Slc39a8* heterozygous knockout mice (HET) and their wild-type (WT) littermates of both sexes were used.

For both the CPP (injections immediately before conditioning trials) and CPA (injections immediately after conditioning trials) studies, 2 g/kg injections of ethanol were paired with tactile floor cues during conditioning sessions, and floor preference/avoidance was then assessed during drug-free tests. We found no effect of genotype on aversive sensitivity to ethanol. Interestingly, HET mice showed significantly higher ethanol CPP expression than WT mice after two weeks of conditioning. This suggests *Slc39a8*-deficiency increases ethanol reward sensitivity without altering aversive sensitivity. In ongoing studies in our lab, we are testing the effects of increasing doses of ethanol on locomotor and anxiety-like behavior in an open field apparatus, which will add important insight on how changes in *Slc39a8* expression impact sensitivity to other effects of ethanol

University of New Mexico

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Poster 36: Regulation of ULK4 expression by the inverted allele of CHRFAM7A in the neuronal and immune tissue

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Human specific gene *CHRFAM7A*, a fusion product between *ULK4/FAM7A* and *CHRNA7*, is present in 99% of human population in different copy number (CN) and orientation. Both the direct and inverted alleles are transcribed with similar frequency, suggesting they underwent similar selective pressure and that both are functional. The direct allele is translated, and the protein incorporates into a7 nAChR modifying its function; the mechanism of the inverted allele is unclear. As the inverted allele (*CHRFAM7A_Δ2bp*) is not translated and its expression does not affect *CHRNA7*, we hypothesized that the *CHRFAM7A_Δ2bp* mechanism is RNA mediated regulation of ULK4. Human brain RNAseq data, brain tissue, primary macrophages (MΦ), and the iPSC model were used to study ULK4 expression and regulation. Analyzing RNAseq data from ROSMAP, we detected the long and short ULK4 transcripts and demonstrated that the long isoform expression correlates with *CHRFAM7A_Δ2bp* CN. qPCR with isoform-specific primers further confirmed that with increasing *CHRFAM7A_Δ2bp* CN, correlation between the isoforms decreases in human brain and MΦ. In the iPSC model, the increased long to short isoform ratio - both at the mRNA and protein level - was detected in neuronal progenitors and MΦ containing *CHRFAM7A_Δ2bp* compared to *CHRFAM7A* null cells. In the inverted line, higher ULK4 Long/ ULK4 Short ratio correlated with higher level of α-tubulin acetylation and polymerization. These findings suggest that the *CHRFAM7A_Δ2bp* exerts genetic epistasis on *ULK4* resulting in increased long to short *ULK4* isoform ratio. Our iPSC model presents an opportunity to elucidate the molecular mechanism of ULK4 regulation.

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Poster 38: Unveiling Novel Histone Modifications in Drosophila Sleep

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Histone monoaminylations represent a novel class of histone post-translational modifications (PTMs), in which monoamine neurotransmitters—such as dopamine, serotonin, and histamine—are covalently attached to the tail region of histone H3 via transamidation reactions. In *Drosophila*, monoaminergic neurotransmission and neural circuits play key roles in regulating sleep and wakefulness. We therefore hypothesized that this novel form of histone modification might play a direct role in controlling sleep in *Drosophila*.

Using a set of *Drosophila* neurogenetics tools, we found that perturbing histone monoaminylation caused nighttime sleep loss, specifically within a defined circadian window, indicating a defect in sleep maintenance. We conducted a large-scale, unbiased, circuit-based screen and identified the cell types that supported this histone monoaminylation-dependent sleep phenotype. Unexpectedly, we discovered that the inhibitory neurotransmitter γ-aminobutyric acid (GABA), which also contains a primary amine group, could similarly modify histone H3 via a transamidation reaction. This novel histone mark, termed histone H3-carboxypropylaminylation, along with other histone monoaminylation marks, exhibited circadian features and regulated sleep/wake behavior in a time-of-day-dependent manner. We employed a comprehensive set of biochemistry, genetic, and genomic approaches to further characterize the gene regulatory network underlying histone monoaminylation-dependent sleep regulation. Because monoamine biochemistry and histone proteins are remarkably conserved between humans and flies, this work may reveal epigenetic mechanisms of sleep regulation that are evolutionarily conserved.

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Poster 40: The Effect of Teneurin-4 on Cue-Reactivity

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Genome-wide association studies have identified teneurin-4 (*Tenm4*) as a potential candidate gene underlying individual variation in drug-related traits, including cue-reactivity. Teneurin-4 produces the peptide Teneurin C-terminal Associated Peptide 4 (TCAP-4), which we hypothesized would alter reward cue-reactivity as tested by Pavlovian conditioned approach (PavCA). Based on prior evidence that another TCAP (TCAP-1) crosses the blood-brain barrier, we chose intravenous administration instead of ICV. Fifty-six female Sprague Dawley rats were implanted with intravenous jugular catheters to administer TCAP-4. Rats were randomly assigned to one of four groups: 0 pmol (n=14), 300 pmol (n=14), 1000 pmol (n=14) or 3000 pmol (n=14). Rats underwent eight daily PavCA sessions. Rats received IV TCAP-4 (300, 1000, or 3000 pmol) or vehicle (0 pmol) 30 minutes before each of five PavCA sessions, followed by three sessions in which TCAP-4 was not given. The main measures in PavCA testing included lever contacts, food cup entries, lever and food cup latency, and PavCA index. Following the last PavCA session, two days of 40-minute conditioned reinforcement tests were conducted to evaluate how well the lever reinforces an instrumental response. TCAP-4 did not produce the expected reduction in cue approach (sign-tracking), although food cup entries (goal-tracking) increased significantly ($p < 0.05$), with the largest increases in early sessions. This suggests a shift away from lever-directed incentive salience rather than a global reduction in learning. The conditioned reinforcement test suggested TCAP-4 reduced reinforcing value of the cue. These results identify *Tenm4* as a potential regulator of individual differences in cue-guided motivation.

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Poster 42: Knockout of mTORC1 modulator, GPR155, reduces ethanol preference in socially-housed female mice

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Alcohol use disorder (AUD) affects 1 in 10 people in the United States alone, causing significantly reduced quality of life in affected individuals. Despite the prevalence and social, economic, and medical burdens associated with AUD, understanding of genetic contributors remains limited. One of the major molecular mechanisms underlying alcohol seeking and reward is mammalian target of rapamycin complex 1 (mTORC1) signaling. mTORC1 is the master cell growth regulator; it is ubiquitously expressed, and it integrates nutrient signals in order to direct cell growth through a general translation mechanism. In the brain mTORC1 is involved in neuronal growth, in part directing formation of learning and memory. Through this process, mTORC1 is thought to encode the rewarding effects of addictive stimuli. In fact, research has found that abnormal mTORC1 activity alters drug seeking, including ethanol seeking. However, the exact mechanism through which mTORC1 affects drug seeking is poorly understood. Here, we investigate the role of a novel modulator of mTORC1 signaling with specific neuronal expression, GPR155, on ethanol-related behavior. We investigated recovery from ethanol-induced sedation as well as ethanol drinking paradigms in a GPR155 knockout mouse model. While we find that motor recovery from ethanol-induced sedation and blood ethanol clearance are unaffected by GPR155 knockout status, ethanol preference is decreased in socially-housed female GPR155 knockout mice. Findings are not only the first to identify the behavioral role of GPR155, but they are also the first to identify its role in ethanol drinking behaviors, identifying it as a novel target of alcohol research.

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Poster 44: Adolescent restraint stress increases adult morphine consumption in a sex- and strain-dependent manner

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Opioid Use Disorder remains an unrelenting public health concern in the United States, with hundreds of overdose deaths occurring daily. Clinical data suggest that women may be more susceptible to the addictive properties of opioids than men. Although adolescent stress is a risk factor for later use, it remains unclear whether biological sex moderates vulnerability to stress-induced increases in opioid use. To address this gap, we investigated how sex and genetic background influence the effects of adolescent

stress on opioid intake. Male and female C57BL/6J and BALB/cJ mice were exposed to repeated restraint stress (2 h/day for 14 days; PND 37 – 50) or control conditions. Body weight was measured daily, and blood corticosterone was obtained at the end of the final restraint session. In adulthood (PND 60), morphine consumption was examined using a two-bottle choice protocol. Repeated daily restraint in adolescence was an effective stressor, as evidenced by delayed body weight gain and elevated corticosterone levels, even after 14 exposures, in both strains and sexes. Adolescent stress selectively increased morphine consumption in female C57BL/6J mice compared to controls, with no significant effects observed in male C57BL/6J or BALB/cJ mice. Our findings highlight how adolescent stress can alter future intake of opioids in a strain and sex specific manner, supporting gene X environment contributions to Opioid Use Disorder.

Poster 46: Hindbrain Circuits Underlying Short-Interval Prepulse Inhibition: Implications for Sensorimotor Gating and Neuropsychiatric Disorders

Hariom Sharma

National Institute of Health

Hariom Sharma1, Noel McGrory1, Giovannina Kirby1, Harold A Burgess1

Abstract: Disruptions in sensory gating contribute to several neuropsychiatric disorders, including schizophrenia, yet the neural mechanisms governing short-interval prepulse inhibition (PPI) remain incompletely understood. PPI is a conserved sensorimotor gating mechanism that relies on precise temporal processing of sensory inputs. Here, we identify a hindbrain circuit that selectively regulates PPI at short inter-stimulus intervals (ISIs) using the Gal4 driver lines y242 and y467. Both lines share overlapping expression in rhombomere 3, revealing this region as a critical locus for short-ISI PPI modulation. Functional analyses demonstrate that neurons within rhombomere 3 specifically regulate PPI at brief ISIs without affecting long-ISI PPI. Within this population, a medially located subpopulation of GABAergic neurons was identified, implicating inhibitory control as a key mechanism. Anatomical tracing shows that rhombomere 3 neurons project directly to the Mauthner (M) cell, a command neuron essential for startle responses. Consistently, the M cell expresses GABA receptor 5, and disruption of this receptor impairs PPI. Together, these findings define a rhombomere 3, Mauthner cell inhibitory pathway that gates sensorimotor responses at short ISIs, providing novel insight into the neural substrates of temporal processing and potential mechanisms underlying sensory gating deficits in neuropsychiatric disorders.

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Poster 48: Manipulation of the autism-related gene, neuroligin 3, reveals a shared genetic basis for social behaviour and aging

Judy Kurbaj

Western University

In humans, *neuroligin 3* (*NLGN3*) is a gene associated with autism, yet its role in behaviour and lifespan remains unclear. Loss of function of *nlg3*, the *Drosophila melanogaster* ortholog of human *NLGN3,* has been shown to disrupt social behaviours, suggesting its involvement in circuits underlying social interaction. To test which genetic pathways were influenced by *nlg3*, we analyzed RNA-seq data from *Drosophila nlg3* loss of function mutants. We found that genes linked to longevity were downregulated, with gene ontology analysis indicating enrichment in aging-related pathways, including key regulators such as *Drosophila* FOXO (dFOXO) and heat shock proteins HSPs (stress-responsive chaperones linked to proteostasis). We manipulated *nlg3* expression using deficiency lines, downregulation, and overexpression using the Gal4-UAS system. RT-qPCR revealed that overexpression of *nlg3* significantly suppressed the expression of genes encoding HSP68 and HSP70; while the expression of dFOXO remained unchanged. In contrast, *nlg3* reduction showed downregulation of the genes encoding dFOXO, HSP68, and HSP70. Behavioural assays showed that *nlg3* overexpression increased social spacing and reduced lifespan, while partial loss of *nlg3* in heterozygous deficiency flies extended lifespan without affecting social behaviour. Furthermore, RNAi-mediated knockdown led to an even stronger lifespan extension, but in that case, with increased social spacing. This supports a dosage-sensitive role for *nlg3* in regulating social behaviour and lifespan in *Drosophila*, with implications for aging and neurodevelopmental disorders in more complex organisms.

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Poster 50: Natural Variation in Olfactory Attraction among C. elegans Wild Strains.

MD Main Udding

University at Buffalo

Smelling diacetyl, a microbial-fermentation byproduct, is a key food cue for *Caenorhabditis elegans*. Chemotaxis assays serve as a traditional model for olfactory behavior. However, the N2 reference strain may not reflect species-wide sensitivity. We first sought to establish straightforward, replicable conditions to enhance the reproducibility of the volatile chemotaxis response, so that we could use the assay to compare diacetyl responses across wild strains. After systematically optimizing hermaphrodite age, lay duration, brood size, and incubation temperature, we found that four gravid day-1 adults and a 16-hour lay at 20 °C produced abundant, synchronized progeny and highly reproducible chemotaxis responses. We established a robust dose–response curve for diacetyl and identified 3×10^{-4} as the dilution that elicited an N2 chemotaxis index (CI) of ~0.6 so that both diminished and elevated CIs relative to N2 could be observed. Applying this protocol to 48 wild strains (the 12 divergent + 36 mapping strains) together with N2 showed that across the 49-strain panel, CIs ranged from approximately 0.20–0.85. Several strains

exceeded N2 while others underperformed, placing N2 in the upper-middle rather than at the maximal end of the species' olfactory spectrum. In sum, our streamlined conditions enable sensitive, comparable measurements across strains and set the stage for genome-wide association (GWA) studies to explore how natural genetic variation between wild strains contributes to differences in olfactory behavior.

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Poster 52: Integrative Multi-Ancestry Genomic and Neuroimaging Analysis of ADHD-Related Behavioral Phenotypes in Children: Interaction-Based GWAS Linking Genetic Variation, Brain Structure, and Symptom Severity

Yashita Arora
University at Buffalo

Maulik Masaliya1 , Dielin Wang2 , Yashita Arora3 , Shuai Wang4 , Giulia Demo5 , Jamal B. Williams6

Background. ADHD is highly heritable, but case-control GWAS explain only a small fraction of that heritability. Genetic variants interacting with continuous behavioral traits may account for some missing heritability. We tested this using interaction-based GWAS across six ADHD phenotypes and structural MRI in a pediatric cohort. Methods. We used the ABCD Study (n up to 8,302 children, ages 9–11), stratified into five ancestry groups. Six phenotypes: hyperactivity, inattention, impulsivity, emotional dysregulation, CBCL Attention Problems T-scores, and Flanker inhibitory control. SNP × behavior interactions were modeled on 34 sMRI measures from the Desikan–Killiany atlas in PLINK and meta-analyzed across ancestries with METAL. Significant loci ($p < 5\times10^{-8}$) collapsed into 1 Mb windows. Results. We identified 589 genome-wide significant interaction loci — substantially exceeding prior ADHD GWAS of comparable sample size. rs56817477 (Chr 2) reached $Z = -7.76$ ($p = 8.7\times10^{-15}$) for 3rd ventricle volume independently in hyperactivity, emotional dysregulation, and inattention, with identical direction across all three analyses — within-study SNP-level replication not previously reported for a structural brain locus in ADHD. Precentral gyrus volume was significant across all five symptom composites, identifying primary motor cortex as a transdiagnostic neuroanatomical hub. Impulsivity yielded 18 independent white-matter hyperintensity loci across 13 chromosomes. Sixty-three ventricular clusters showed convergence across three or more phenotypes. Interaction effect directions reversed across phenotypes, revealing phenotype-specific genetic architectures. Conclusions. SNP-by-behavior interactions expose ADHD neurobiology invisible to case-control designs. Phenotype-specific pathways converge on ventricular, sensorimotor, and fronto-parietal systems. The chromosome 2 locus is the most robustly replicated structural brain locus in pediatric ADHD genetics to date.

Keywords: ADHD · interaction GWAS · structural MRI · SNP×behavior · Desikan–Killiany · ABCD Study · ventricular volume · precentral gyrus · white matter

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Date: Wednesday, 10/June/2026

7:30am - 8:00am	**Registration** Location: **Foyer at Assembly Room entrance**
8:00am - 10:00am	**Selected Talks 1** Location: **Assembly Room/Ballroom** Session Chair: **Megan Mulligan** Session Chair: **Justine Anne Guevarra** Session Chair: **Andre Lucas Borges** Session Chair: **Markos Chatzigiannis**

From perception to valence: Fox neurons assign state-dependent valence to nutrient taste cues in Drosophila

Lisha Shao
University of Delaware

Kevin Christie[1#], Tarandeep Dadyala[1#], Irina Sinakevitch[1], Nicholas Collins[1], Phuong Chung[2], Masayoshi Ito[2,3], Lisha Shao[1,*]

Assigning valence—appeal or aversion—to gustatory stimuli and relaying it to higher-order brain regions to guide flexible behaviors is crucial to survival. Yet the neural circuits that transform taste into motivationally relevant signals remain poorly defined in any model system. In *Drosophila melanogaster*, substantial progress has been made in mapping the sensorimotor pathways encoding intrinsic valence for feeding and the architecture of the dopaminergic reinforcement system. However, where and how "effective" (i.e., real-time) valence is first imposed on a taste has long been a mystery. Here, we identified a pair of subesophageal zone interneurons in *Drosophila*, termed Fox, that impart reinforcing positive valence to sweet taste and convey this signal to the mushroom body, the fly's associative learning center. We show that Fox neuron activity is necessary and sufficient to drive appetitive behaviors and can override a tastant's intrinsic neutral or aversive valence without impairing taste quality discrimination. Furthermore, Fox neurons relay the positive valence to specific dopaminergic neurons that mediate appetitive memory formation. Our findings reveal a circuit mechanism through which effective valence is bestowed upon sweet sensation and transformed into a reinforcing signal that supports learned sugar responses. Preliminary data further suggest that Fox function may extend beyond sweetness: Fox may amplify real-time valence for the tastant most valuable to the animal's current physiological state, including during sugar–protein choice. Fox neurons thus form a convergent–divergent "hourglass" circuit motif, acting as a bottleneck for valence assignment and distributing motivational signals to higher-order centers. This architecture confers both robustness and flexibility in reward processing—an organizational principle that may generalize across species.

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Specialized enhancer activity associated with convergent evolution of vocal learning

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Carnegie Melon University

Rajee Ganesan 1 , Andrew Wang 2 , BaDoi N. Phan 2,3 , Michael J. Leone 2,3 , Heather Sestili Harper 2 , Andreas Pfenning 2,4

Vocal production learning is the ability to imitate sounds through social exposure. The rare trait is believed to have independently evolved three times in birds and five times in mammals, a prime example of convergent evolution. All studied vocal learning species have evolved a specialized forebrain sensorimotor learning circuit that is either absent or rudimentary in their closer, vocal non-learning relatives. Previous work within the lab has found that the "regulatory code" linking genome sequence to cell-type-specific function is highly conserved across mammals. In this study, we leverage this principle to identify candidate enhancers from publicly available Zoonomia datasets, and trained machine learning models predicting open and closed chromatin across all 240 mammalian motor cortex regions. We screened for differences in regulation between vocal learning and non-learning species by applying the Tissue-Aware Conservation Inference Toolkit, a machine learning approach to study how enhancer activity conservation relates to phenotype evolution. These machine learning models can learn sequence patterns of enhancers that robustly predict conserved activity across evolutionary distances, allowing us to test whether enhancer conservation patterns are associated with the evolution of vocal learning. Our results revealed lineage-specific gains and losses of regulatory elements, and we show that L6 corticothalamic neurons and oligodendrocytes had the strongest enrichment with the vocal learning phenotype, suggesting key roles in vocalization-related traits through motor learning and synaptic plasticity. Future studies will include single cell integration across species to identify orthologous cell populations and to better understand molecular mechanisms associated with the evolution of vocal learning.

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In vivo brain imaging and ex vivo permeability assays support a BBB mechanism underlying increased brain oxymorphone levels in Zhx2 knockout females following oxycodone administration

Camron Bryant
Northeastern University

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Oxycodone (OXY; active ingredient of OxyContin®) is a major contributor to the opioid epidemic. We genetically mapped and validated loss-of-function in zinc-fingers and homeobox 2 (Zhx2) underlying increased brain oxymorphone (OMOR) in female mice. OMOR, an OXY metabolite, is a much more potent and efficacious mu opioid receptor agonist that could increase OXY addiction risk. Transcriptome analysis of Zhx2 knockout (KO) brains via bulk RNA-seq identified enrichment of extracellular matrix, endothelial cells, and cell-to-cell adhesion, suggesting Zhx2 KO compromises blood brain barrier (BBB) integrity. In support, there was a significant reduction in transcript levels of the BBB marker Claudin5 in KO females. Furthermore ex vivo analysis indicated increased permeability of sodium fluorescein but not Evans blue, specifically in hippocampus of KO females, suggesting brain region-dependent disruption of BBB. In vivo structural imaging revealed reduced water diffusion throughout the brain of KO females and enlarged ventricles. In response to OXY in awake mice, KO females showed increased OXYinduced negative bold signal in midbrain and increased OXY-induced positive bold signal in brainstem. Functional connectivity analysis identified decreased brain-wide connectivity in Zhx2 KOs. In addition to a BBB mechanism, KO females also showed increased plasma [OMOR] following systemic OXY, suggesting increased liver metabolism of OXY also contributes to increased brain [OMOR]. To summarize, multiple lines of evidence support a BBB mechanism underlying increased brain [OMOR] in Zhx2 KO females. We are currently conducting functional enzymatic assays of liver microsomes to determine whether increased liver OMOR production also contributes to the phenotype.

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Genomic associations with 24-hour food and fluid intake in heterogeneous stock rats

Nana Amissah
University at Buffalo

Nana K. Amissah[1], Christopher P. King[1], Sydney David[1], Destiny Brakey[2], Luke T Hannan[1], Oksana Polesskaya[3], Quinn Carroll[2], Thiago Missfeldt Sanches[3], K. Linnea Volcko[2]; Apurva Chitre[3]; Denghui Chen[3], Maggie Postolache[2], Hannah Bimschleger[3], Jianjun Gao[3], Khai -Ming Nguyen[3], Beverly Peng[3], Riyan Cheng[3], Leah C. Solberg Woods[4], Abraham A. Palmer[3, 5], Derek Daniels[1,2], Paul J. Meyer[1]

Maintaining fluid homeostasis is critical for life. Although there are individual variations in the behavioral regulation of fluid homeostasis in rats, the source of these variations is poorly understood. To address this, we conducted a genome-wide association study (GWAS) in 826 male and female heterogenous stock (HS) rats examining multiple phenotypes related to 24-hour food and water intake. Rats were housed in hanging wire cages for 24 hours. Total food intake over the 24-hour test was measured, and drinking was measured via a contact lickometer with millisecond resolution and were subjected to GWAS analyses. Total water intake had moderate genetic correlation with total food intake (r_g = .533), and licks (r_g = .565). There was moderate heritability for traits such as mean licks per burst (h^2 = .261), total water intake (h^2 = .224), and burst number (h^2 = .241). Eight unique loci on chromosomes 1, 2, 7, 12, 14 and 20 were associated with several measures of food and water intake. The locus on chromosome 1 was linked with burst number and mean licks per burst. This locus contained the candidate gene *Stx11* which mediates lipid metabolism (Zhang et al., 2022). The locus on chromosome 2 was associated with water intake, and contained the candidate gene *Syt6,* which is involved in synaptic modulation thorough the brain derived neurotrophic factor (Wong et al., 2015). Food intake linked to a locus on chromosome 14, and contained

Paqr3 a gene that regulates glucose and lipid metabolism disorders caused by insulin resistance. These candidate genes were identified by examining eQTL and coding variants. These results demonstrate that the individual differences in food and fluid intake have genetic components. Further studies will examine causal links between the identified candidate genes and ingestive behaviors by directly manipulating these genes using CRISPR-mediated approaches.

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Dissecting the strain and sex specific connectome signatures of unanesthetized C57BL/6J and DBA/2J mice using magnetic resonance imaging

Helen Kamens

Penn State University

Helen M. Kamens[1], Tanzil M. Arefin[2,3,4,5,6], Hayreddin Said Unsal[3,7], Thomas Neuberger[2,3], Nanyin Zhang[2,3,4]

Mouse models are an essential tool for understanding behavior and disease states in neuroscience research. While genetic and sex-specific effects have been reported in many neurodegenerative and psychiatric illnesses, these factors may also alter baseline neuroanatomical features of mice. This raises the question of whether the observed changes are related to the disease being studied (i.e., pathological differences) or if there are baseline strain or sex differences that may potentially predispose animals to different responses. Over the past decade, tremendous effort has been made in mapping neural architecture at various scales; however, the complex relationships including identifying genetic and sex-specific differences in brain structure and function remain understudied. To bridge this gap, we used C57BL/6J and DBA/2J mice, two of the most widely used inbred mouse strains in neuroscience research, to investigate strain and sex-specific features of the brain connectome in awake animals using magnetic resonance imaging (MRI). By combining resting-state fMRI and diffusion MRI, we found that the motor, sensory, limbic, and salience networks exhibit significant differences in both functional and structural domains between C57BL/6J and DBA/2J mice. Further, functional and structural properties of the brain were significantly correlated in both strains. Our results underscore the importance of considering these baseline differences when interpreting the brain-behavior interactions in mouse models of human disorders.

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Using a Connectome to Identify Motivational Neurons for Specific Conflict Resolution

Adrian Rothenfluh

University of Utah

Perham Black, Kelcey Stapleton, Geanette Lam, Aylin Rodan, *Adrian Rothenfluh

Animal life is full of daily decision making. These are easy when rewards are available at no cost or peril. However, many situations require a weighing of the cost vs. benefits of specific decisions. To model decisions that require a cost/benefit analysis, we have established a novel assay where *Drosophila* choose between a small reward (eg. 10mM sucrose) at little cost (liquid solution) and a larger reward (30mM sucrose) at a higher cost (food embedded in agarose, which requires work to get the sucrose out). We find that the internal state of a fly (eg. food deprivation) will motivate them to prefer the high-reward/high-cost option over the low/low one. Silencing ~half of the flies' brain dopamine neurons causes them to show the same motivation for sucrose, but reduced motivation to 'work for' amino acids when amino acid-deprived. In lieu of a classical anatomical screen, we have simulated this situation in the connectome-derived virtual brain and screened for dopaminergic neurons that affect this cost/benefit

calculation. We focus on 2 sets of *in silico*-identified DA neurons and predicted that one set will be involved in signaling satiety but will not alter the hi reward+cost vs. low reward+cost calculation. For the other set of DA neurons, we predicted the opposite result. Manipulating these two sets of DA neurons *in vivo* confirmed our prediction. We thus identify: 1] a set of DA neurons mediating satiety (anti-motivation). 2] a second set that is specifically involved in motivating flies to consume amino acids at the cost of work (hard food), but not of bitterness (aversive due to the potential cost of toxicity). 3] the value of using a virtual brain simulation to find motivation-relevant neurons *in vivo*. We next seek to understand the cellular and molecular correlates of the motivated state in these neurons.

Huntsman Mental Health Institute, Dept. Psychiatry, University of Utah, SLC.

10:00am - 10:30am

Break
Location: **Assembly Room**

10:30am - 12:30pm

Symposium 3: Dissecting the Development of Opioid Use Disorder Using Cross-species Systems Genetics Approaches

Location: **Assembly Room/Ballroom**
Session Chair: **Marissa Ehringer**

Initial QTL Mapping of Oral Oxycodone Self-Administration in the Hybrid Rat Diversity Panel

Hao Chen

University of Tennessee Health Science Center

Hao Chen1, Shuangying Leng1, Jun Huang1, Caroline Jones2, Robert W Williams2, and Burt M Sharp2

Most individuals affected in the national epidemic of oxycodone abuse began taking oral oxycodone by prescription. We studied vulnerability to oxycodone intake in a rat model of oral drug self-administration (SA) under a fixed ratio 5 schedule, where licking was used as the operant behavior. Rats were not water or food deprived. Training started with 0.025 mg/ml oxycodone, gradually increased to 0.1 mg/ml, and session length was extended from 1-h to 16-h, followed by extinction and reinstatement sessions. Females (49 strains) and males (45 strains) licked significantly more on the active spout compared to the inactive spout ($p<0.001$). The number of active licks were greater in females than males during 4-h and 16-h sessions ($p<0.001$ for all). Both sexes escalated intake during 16-h extended access vs 4-h sessions ($p<2e-16$). The heritability of active licks has a range from h2 of 0.22 to 0.59, while that for inactive licks ranged from 0.08, 0.34 at different stages of self-administration. Initial QTL mapping using GEMMA with LOCO identified several significant loci, among them, a region in Chr 1 between 159-172 Mb was associated with oxycodone intake at 0.025, 0.05 and and 0.1 mg/ml, 4h sessions, with max – log10(p) values of 6.1, 5.1 and 5.6, respectively. Potential candidate genes within this range include Cyp2r1 and Pde3b, both have strong cis-eQTL in the brain and are involved in vitamin D metabolism.

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Funding provided by NIH/NIDA U01DA053672.

Genetic and neurobiological correlates of opioid use disorder vulnerability and resiliency using a rat model

Brittany Kuhn

Baylor University

BN Kuhn1 (presenting author underlined)

The rise of opioid use disorder (OUD) worldwide makes it imperative to disentangle the behavioral, genetic and neurobiological correlates associated with both OUD vulnerability and resiliency. Using a novel preclinical rat model of OUD that captures the multi-symptomatic diagnosis and complex multidimensional interactions between symptoms conferring OUD propensity, we have shown distinct behavioral and neurobiological profiles associated with each phenotype (n>1000). Additionally, genome-wide association study (GWAS; n=874) analysis indicates both resiliency and vulnerability to OUD are heritable states. GWAS identified genetic variants for nociception, heroin consumption and motivation to obtain heroin, with OUD vulnerability associated with the latter two. Several of the identified genes are known regulators of neuroplasticity, thereby prompting further investigation into neuroplastic mechanisms contributing to OUD propensity. Guided by findings from GWAS, we are assessing OUD phenotypic differences in components of the extracellular matrix (ECM), microglia and dendritic spine morphology within a canonical circuit necessary for OUD-like behaviors (prelimbic cortex, PrL; nucleus accumbens core, NAc; ventral pallidum, VP). Opposing phenotypic differences in PrL and VP ECM and microglia plasticity are evident, suggesting a mechanistic role for these neuroplastic components in mediating OUD vulnerability and resiliency. Furthermore, cell-specific alterations in NAc dendritic spine morphology are currently underway. Together these data identify novel genetic loci associated with OUD behaviors and vulnerability which further guided the assessment into neuroplastic measures that are likely contributing to OUD vulnerability and resiliency.

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Voluntary oxycodone self-administration reveals genetic variation in analgesic tolerance and hyperalgesia in rats

Tolulope Ajanaku

University of Colorado Boulder

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Prescription opioid use is limited by the development of analgesic tolerance and opioid-induced hyperalgesia (OIH), yet the extent to which these adaptations are shaped by genetic background versus drug exposure remains unclear. Here, we used 20 inbred Hybrid Rat Diversity Panel (HRDP) strains to quantify strain differences in baseline thermal sensitivity, oxycodone analgesia, the development of tolerance, and OIH following voluntary oxycodone self-administration. Rats completed a tail immersion test before (Pre-SA, before self-administration) and after (Post-SA, after self-administration) intravenous oxycodone or saline self-administration. Analgesia was summarized as the area under the withdrawal-latency curve, with tolerance defined as the change in area under the curve between trials. Heritability was estimated from the mixed-effects models of various phenotypes. Baseline and post-exposure analgesia and thermal sensitivity were moderately heritable ($H^2 \approx 0.24–0.30$), whereas tolerance and change in thermal sensitivity showed much lower heritability ($H^2 \leq 0.10$), indicating a larger contribution of non-genetic factors to these adaptations. Most strains exhibited classic tolerance to oxycodone, but a few showed sensitization or resistance. Most strains also displayed increased thermal sensitivity after oxycodone self-administration, indicative of OIH. Surprisingly, total oxycodone intake was only weakly related to tolerance at both individual- and strain-mean levels, suggesting that the mechanisms regulating oxycodone consumption and those governing analgesic tolerance are at least partly dissociable. Together, these findings indicate that opioid analgesia and baseline pain sensitivity are strongly shaped by genetic background, whereas tolerance and OIH that emerge following volitional oxycodone intake are less heritable and loosely related to total drug exposure.

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Sex-specific Concordance of Striatal Transcriptional Signatures of Opioid Addiction in Human and Rodent Brains

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Micah A. Shelton[1], Nicole Horan[1], Xiangning Xue[2], Lisa Maturin[3], Darrell Eacret[4], Julie Michaud[5], Navsharan Singh[6], Benjamin R. Williams[7], Mackenzie C. Gamble[6,7], Joseph A. Seggio[5], Madeline Kuppe-Fish[7], BaDoi N. Phan[8], George C. Tseng[2], Julie A. Blendy[4], Leah C Solberg Woods[9], Abraham A. Palmer[3], Olivier George[3], Marianne L. Seney[1*], Ryan W. Logan[7,10*]

Opioid use disorder (OUD) has emerged as a severe, ongoing public health emergency. Current, frontline addiction treatment strategies fail to produce lasting abstinence in most users. This underscores the lasting effects of chronic opioid exposure and emphasizes the need to understand the molecular mechanisms of drug seeking and taking, but also how those alterations persist through acute and protracted withdrawal. Here, we used RNA sequencing in post-mortem human tissue from males (n=10) and females (n=10) with OUD and age and sex-matched comparison subjects. We compared molecular alterations in the nucleus accumbens (NAc) and dorsolateral prefrontal cortex (DLPFC) between humans with OUD and rodent models across distinct stages of opioid use and withdrawal (acute and prolonged) using differential gene expression and network-based approaches. We found that the molecular signature in the NAc of females with OUD mirrored effects seen in the NAc of female mice at all stages of exposure. Conversely, males with OUD showed strong overlap in expression profile with rats in acute withdrawal. Co-expression networks involved in post-transcriptional modification of RNA and epigenetic modification of chromatin state. This study provides fundamental insight into the converging molecular pathways altered by opioids across species. Further, this work helps to disentangle which alterations observed in humans with OUD are driven by acute drug exposure and which alterations are consequences of chronic exposure.

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12:30pm - 1:30pm	**Lunch** Location: **Lower Lounge/Ballroom**
1:30pm - 3:30pm	**Symposium 4: Functional Implications of co-transmission in Regulating Neuroplasticity and Behavior** Location: **Assembly Room/Ballroom** Session Chair: **Lewis Sherer** **A Co-Transmitting Neuron Regulates Aggression Through Pre- and Postsynaptic Mechanisms** **Lewis Sherer** Brown University **Experience-Dependent Co-Transmission Shapes Thermosensory Navigation** **Andrea Cuentas-Condori** Yale School of Medicine **Sex-specific mechanisms of dopamine neuron resilience across species** **Samuel Mabry** University of Pittsburgh **Separating glutamatergic and dopaminergic subtypes in motivated behavior** **David Root** University of Colorado Boulder
3:30pm - 4:00pm	**Break** Location: **Assembly Room**
4:00pm - 5:00pm	**Plenary 3: Plenary 3: Early Career Achievement Award Lecture (Assoc.Professor Chongyuan Luo)** Location: **Assembly Room/Kurtzman Room** Session Chair: **Francesca Telese** **Dissecting Human Brain Development and Neuropsychiatric Disorders with Single-Cell and Spatial 3D-Multiomics** **Chongyuan Luo** University of California San Diego
5:00pm - 5:30pm	**Additional Selected Talk** Location: **Assembly Room/Ballroom** Session Chair: **Paul Meyer** **Binary partitioning of human brain organization due to divergent human cytoskeletal evolution** **Kinga Szigeti** SUNY at Buffalo
5:30pm - 7:00pm	**Posters B: Poster Session B** Location: **Assembly Room/Kurtzman Room** **Poster 1: 5-HTR2 serotonin receptor subtypes bidirectionally modulate acoustically-evoked behavior selection in zebrafish** **Rebecca Voss** Haverford College Rebecca Voss[1], Rebecca Osbaldeston[2], Matt Curran[2], Kevin Villafañe[2], Cole Roland[2], Roshan A. Jain[1,2] Serotonin (5-HT) regulates many aspects of behavior including mood, sleep, appetite, social interactions, and decision-making. A major challenge in untangling serotonin's many distinct functions in humans is determining which of its 14 receptors are responsible for these diverse processes. We are modeling serotonin's decision-making role through a simple response selection of zebrafish larvae, where serotonin modulates how zebrafish larvae bias their selection between two different acoustically evoked escape behaviors: an explosive short-latency response (SLC) and a kinematically and neuronally distinct long-latency response (LLC). Through a pharmacological screen, we found that 5-HT2B/2C receptor agonists shift behavioral bias towards SLC responses and antagonists shift bias towards LLC responses. To distinguish which specific receptors drive this modulation of decision-making, we used CRISPR-directed mutagenesis to disrupt each of the three zebrafish genes encoding 5-HTR2B/C subtypes, and then assessed the behavioral impacts. Mosaic larvae in which *htr2cl1* was disrupted showed a behavioral shift towards LLC response, consistent with the drug data. In contrast, disrupting *htr2b* and *htr2cl2* produced the opposite shift in escape behavior selection bias phenotype, towards SLC responses. Because mosaic G0 individuals may vary in the degree and type of molecular disruption, we have generated a set of novel germline-transmitted *htr2b*, *htr2cl1*, and *htr2cl2* mutations. We are now using this set of mutations to clarify

the individual and combined roles of 5-HTR2 receptors in simple acoustic decision-making. Together, our pharmacological and genetic results support a model in which 5-HT2B and 5-HT2C receptors bidirectionally modulate vertebrate decision-making following acoustic threat.

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Poster 3: A non-additive polygenic genetic architecture underlies the divergence of male courtship song type in Drosophila

Helena Gifford

University of Pennsylvania

H Gifford1, S Lin1, Y Ding1

From the vibrant dances of Birds of Paradise to the smelly nuptial gifts of dung beetles, mating signals have incredible diversity across the animal kingdom. The evolution of mating signals is a question with major implications for speciation and sexual isolation. *Drosophila* courtship song is an innate behavior where males vibrate their wings to produce context-dependent and species-specific acoustic signals for potential female mates. We found that the hybrid offspring of *D. teissieri* and *D. santomea*, two closely related species that sing distinct song types, produce “chimeric” courtship songs. These chimeric song types consist of simultaneous production of different parental song elements used for similar social contexts, suggesting that the species divergence of song types lie in the motor patterning circuits downstream of courtship song decision-making nodes. The genetic differences underlying this change are unknown. To explore the genetic differences, we generated a backcross F2 population of 570 individuals, scored song phenotypes, and used multiplexed shotgun genotyping to genotype the population. Quantitative trait locus mapping reveals a highly polygenic basis underlying the divergence in courtship song traits. Intriguingly, we also uncover pervasive inter-chromosomal epistasis, with any song phenotype effect being highly contingent on the extent of *D. teissieri* chromosomal introgression into the *D. santomea* genetic background. Our results indicate that species divergence in courtship song types involves changes at many gene loci that work together in a highly non-additive manner to shape the evolution of motor patterning circuits, highlighting the complexity of the genetic basis for neuronal and behavioral evolution.

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Funding Support: NIGMS Grant GM142678

Poster 5: Modeling Behavior in Modern Day Neuroscience: Statistical Inference Using Generalized Linear (Mixed) Models.

Antonio Marini-Davis

University of Maryland, Baltimore County

Antonio Marini-Davis and Fernando J. Vonhoff.

Datasets in the field of neurobiology are becoming increasingly complex, and common statistical methods such as T-test and ANOVA are struggling to capture this complexity. Researchers often discard informative details of their data through use of improperly specified models or non-parametric tests to conclude treatment effects. I detail here the increasingly used method of generalized linear (mixed) models to make more precise statistical conclusions when data doesn’t fit the normal assumptions for traditionally used statistical tests. Using a real behavioral dataset generated using a two choice preference based assay using *Drosophila Melanogaster*, I delineate the process of recognizing when more complex modeling is necessary, and show the diagnostic tools to ensure proper conclusions are drawn when using model-based statistical inference. Proper model specification allows future researchers to retain valuable information in their data and make better informed conclusions about their experimental outcomes.

University of Maryland, Baltimore County. US

Poster 7: A novel task to evaluate episodic-like memory changes with aging across the lifespan in marmosets

Takeshi Murai

University of Pittsburgh

Takeshi Murai[1], Lauren Mongeau[1], Lauren Bailey[1], Abbey Setlik[1], Stacey J. Sukoff Rizzo[1,2]

Episodic memory is one of the long-term memories that involves the recollection of personal experiences or events (“What”), linked directly with the time (“When”) and place (“Where”). This is also one of the earliest cognitive domains that are impaired in age-related neurodegenerative disorders such as Alzheimer’s disease. We have been establishing a comprehensive battery of touchscreen-based tasks in marmosets that captures a spectrum of cognitive domains sensitive to detect aging-related cognitive decline. The present study describes the establishment of a Paired Associates Learning (PAL) test, which has been successfully used to evaluate episodic-like memory in humans. Initially, marmosets are trained on an FR-1 schedule of positive reinforcement using touchscreens mounted to their home cages. For the PAL task, subjects were trained to associate touch with a specific stimulus (“What”) presented in a specific location (“Where”) to receive a reward. Baseline testing presented two unique stimulus-location pairs. Once the subject learned the stimulus-location combinations, they were assessed in probe trials at 1 day, 2 weeks, 4 weeks, 3 months and 6 months of post-training. Our preliminary data demonstrate that marmosets have the ability to retain intact episodic-like memory over time. Interestingly, we observed the earliest decay in episodic-like memory in aged marmosets within 3 months, while younger marmosets

retain episodic-like memory beyond 6 months. Ongoing studies continue to evaluate natural decay of episodic-like memory in this task in aged versus young subjects with longitudinal studies planned as annual assessments throughout the subject's lifespan.

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Funding Support: National Institute on Aging NIA R24AG073190, NIAU19AG074866

Poster 9:Combining Machine Learning and Multiplexed, In Situ Profiling to Engineer Cell Type and Behavioral Specificity

Robert van de Weerd

Carnegie Melon University

Robert van de Weerd

Neural circuit interventions hold promise for treating nervous system disorders but are limited by lack of specificity, inadvertently affecting nearby neurons and causing side effects. Cis-regulatory elements (CREs) offer a promising strategy to restrict optogenetic or chemogenetic tool expression to specific neuron subtypes. However, CRE discovery faces significant challenges including low *in vivo* success, species-specific activity, difficulties with multiplexed AAV screening, and limited spatial resolution. Here, we developed ESCargoT (Engineered Specificity of Cargo Transcription), a platform combining machine learning-guided CRE prioritization, modular AAV assembly, and multiplexed *in situ* spatial screening focused on the spinal cord, a critical region for pain and itch. First, we trained CRE prioritizing ML models on cross-species chromatin accessibility data from 15 dorsal horn neural subtypes and identified Excit-1, enabling chemogenetic inhibition that reversed inflammatory mechanical allodynia. Subsequently, we accelerated CRE discovery by developing SPRA (spatial parallel reporter assay) for multiplexed screening of a 27-candidate library, identifying new candidates that drive cargo expression in oligodendrocytes and several neuronal subtypes. We then validated two CREs targeting Exc-LMO3 and Exc-SKOR2 neurons and demonstrate that the Exc-SKOR2 enhancer, unlike Excit-1, suppressed chemical itch in mice. Together, our platform enables multiplexed *in vivo* enhancer profiling that accelerates CRE discovery and gene therapy development.

Carnegie Melon University

Poster 11: Sex-Specific Effects of Malat1 and Neuroinflammation in Alcohol Consumption

Daniela Gil

University of Pittsburgh

DV Gil[1], C Ferguson[2], M Miskanic[2], SS Mrozowski[2], GE Homanics[2,3], SP Farris[2,4]

Alcohol activates the neuroimmune system, triggering inflammatory signaling thought to promote alcohol consumption and contribute to alcohol use disorder (AUD). However, the molecular mechanisms linking neuroinflammation to drinking behavior remain unclear. *Malat1* is a widely expressed, evolutionarily conserved long non-coding RNA that promotes pro-inflammatory signaling, including in the central nervous system. *Malat1* is upregulated in post-mortem brains from AUD subjects and rodents chronically exposed to alcohol, yet its causal role in regulating alcohol consumption remains unexplored. We hypothesized that *Malat1* promotes immune-induced increases in alcohol consumption. To assess whether modulation of *Malat1*-associated neuroinflammatory signaling alters alcohol intake, tamoxifen-inducible *Malat1* homozygous floxed, hemizygous CreERT2 (*Malat1* global KO) mice and Cre negative littermate controls (WT) underwent ten days of baseline two-bottle choice (2BC; 10% v/v), followed by ten additional days of drinking post-treatment. One cohort received a single lipopolysaccharide (LPS; 1 mg/kg, i.p.) injection to induce inflammation before this period; another received daily quercetin (30 mg/kg, i.p.), an anti-inflammatory compound reported to regulate *Malat1* function and attenuate alcohol reward, prior to each post-treatment session. A subset of mice from each group was monitored with sipper devices to characterize temporal drinking patterns. LPS increased alcohol intake in females regardless of genotype. Quercetin produced a treatment-by-genotype interaction in females, suggesting *Malat1* differentially modulates quercetin's effects on drinking. Neither treatment altered male drinking, though *Malat1* global KO males consistently consumed more alcohol than WT controls. Together, these findings suggest greater female sensitivity to inflammation-associated drinking, and a sex-specific role for *Malat1* in mediating alcohol intake.

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Funding Support: NIAAA F31 AA032172, NIAAA U01 AA020889, NIAAA R01 AA030257

Poster 13: Variation in Mushroom Body Morphology in Cocaine Preferring Drosophila Genetic Reference Panel Lines

Alp Mete Ummet

Clemson University

Alp M. Ummet [1], Trudy F. C. Mackay[1], Robert R. H. Anholt[1]

Previous studies revealed natural genetic variation in cocaine consumption and preference among the wild-derived, inbred lines of the Drosophila Genetic Reference Panel and implicated the mushroom bodies, brain structures that mediate experience-dependent behavior (Highfill et al., PLoS Genet. (2019) 15, e1007834). Previous studies also showed correlations between variation in mushroom body structure and behavior such as aggression and sleep. (Zwarts et al., Nat. Commun. (2015) 6, 10115). To assess whether variation in mushroom body morphology is correlated with variation in cocaine preference, we conducted an initial study by selecting six DGRP lines, three cocaine-preferring lines in which at least one sex showed preference for a cocaine-supplemented sucrose solution over control solution and three lines with mean aversion scores for cocaine preference. We dissected brains and stained mushroom bodies from males and females separately with an anti-fasciclin-II antibody. We quantified three-dimensional morphometrics of the alpha and beta lobes by confocal microscopy. We also observed the absence of lobes, bilateral asymmetry, and anatomical abnormalities. Our initial studies showed variation in mushroom body morphology and suggested a correlation of cocaine preference with alpha lobe structure and asymmetry. To consolidate these observations with statistical significance, we will expand this initial study to a larger sample of 48 lines. Based on evolutionary conservation of fundamental biological processes, correlations between variation in mushroom body morphology and cocaine preference in the fly brain raise the possibility that subtle variations in neural circuitry in the human brain could contribute to risk for cocaine use disorder.

1 Institute for Human Genetics, Department of Genetics and Biochemistry, Clemson University, Greenwood SC, USA.

Funding Support: Supported by grant DA041613 from the National Institute on Drug Abuse (NIDA) to TFC Mackay and RRH Anholt.

Poster 15. Primary human macrophages as an ex vivo model for human-specific neuroinflammation

Andras Szabados
State university of New York at Buffalo

Andras Szabados, Kateryna M. Dukh, Ivanna Ihnatovych, Kinga Szigeti

α7nAChR is a key element of cholinergic anti-inflammatory pathway (CAIP); its activation leads to the inhibition of NF-κB complex. *CHRFAM7A*, a human-specific gene detected in 99% of the human population is present in different copy numbers and orientation (direct, inverted). The direct allele of *CHRFAM7A* is translated and gets incorporated into the α7 nAChR. The inverted *CHRFAM7A* allele is not translated and is a functional null from the α7 nAChR perspective. We have previously shown that the direct allele prolongs NFKB presence in the nucleus in iPSC derived microglia-like cells. We performed a human macrophage ex vivo study (N=70) to characterize NFKB translocation and Il-6 expression on the CHRFAM7A genetic background.

Primary human macrophages were treated with TLR agonists LPS, imiquimod, PAM2CSK4. NF-κB translocation dynamics was quantified over time using Manders' coefficient. IL-6 expression was measured by ELISA.

In the iPSC model, the direct isogenic MGL cells demonstrated prolonged NF-κB nuclear translocation compared to null in response to LPS. In the human ex vivo model TLR agonists induced NF-κB translocation was prolonged compared to the null MGL on all 3 genetic background: translocation to LPS 4h (direct), 6h (heterozygous), up to 24 h (inverted); response to PAM2CSK4 - 45 min (direct), 2h (heterozygous), 4h (inverted). No NF-κB translocation was detected in response to imiquimod. The level of IL-6 was significantly elevated in response to all treatments, including imiquimod. The presence of both the direct and inverted *CHRFAM7A* alters the immune response to TLR agonists likely via distinct mechanism.

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Funding Support: Community Foundation for Greater Buffalo (Kinga Szigeti).

Poster 17: Inverted CHRFAM7A Allele Enhances Microtubule Dynamics via ULK4 Dependent Mechanisms in the Human Brain

Nicolas Rosas
University at Buffalo

Nicolás Rosas[1], Ivanna Ihnatovych[1], Kinga Szigeti[1]

Human-specific genes contribute to the unique structural and functional features of the human brain. *CHRFAM7A* is one such gene, created by the fusion of the α7 nicotinic acetylcholine receptor (α7nAChR) and ULK4, a member of the ULK kinase family. CHRFAM7A has been associated with neuropsychiatric diseases.

Three CHRFAM7A alleles exist in the human population: the ancestral allele (0-copy), allele harboring the direct-orientation fusion gene (CHRFAM7A), and the allele with inverted fusion gene carrying a 2-bp deletion in exon 6 (CHRFAM7AΔ2bp). Because animal models lack CHRFAM7A and its variants, studying its role in brain development and disease has been challenging. We developed a human isogenic iPSC model to elucidate the function of CHRFAM7A. We have previously shown that the direct allele modulates α7nAChR activity.

Here, we use iPSC-derived medial ganglionic eminence (MGE) neuronal progenitors and primary human monocytes to investigate how the inverted CHRFAM7AΔ2bp allele influence neuronal development and decipher the underlying genetic mechanism. Through quantitative live-cell imaging, we assessed

cytoskeletal debundling, growth cone dynamics, cell motility, and neurite arborization to characterize cellular processes affected by this human-specific variant.

We found that the inverted CHRFAM7A allele modulates ULK4 expression through a distinct genetic epistasis, driven by the emergence of an alternatively spliced ULK4 exon 12-18sh variant. The splice variant shifts ULK4 isoform balance toward the long isoform, leading to a microtubule cytoskeleton gain-of-function that enhances neuronal complexity and cell motility, ultimately strengthening functional connectivity in the human brain.

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This work is supported in part by the Community Foundation for Greater Buffalo (Kinga Szigeti).

Poster 19: Blue light-induced oxidative stress alters dopaminergic function in Drosophila melanogaster

Nour Selim
California State University, Fresno

Nour Selim 1, Romeo Aiyabei 1, Ivan Soto 1, Gauri Paul 1, and Cynthia T. Hsu 1

Parkinson's pathology, in which the progressive loss of dopamine neurons contributes to debilitating loss of motor control, is an important and active area of biomedical research. While oxidative stress is known to contribute to neurodegeneration, the potential for environmental influences to exacerbate the condition are not well understood. Blue light is a prevalent environmental factor that is attributed to the habitual use of electronics; as such, exploring its adverse effects on humankind is a necessity. Previous literature has shown that blue light induces oxidative stress in *D. melanogaster*, which may indicate the risk for increased damage to DA neurons and perpetuate the loss of motor functions. We have found that blue light exposure not only increases oxidative stress but also sleep in a cryptochrome dependent manner. This raises two questions that we are currently investigating: (1) if blue light is sufficient to increase oxidative stress specifically in the dopaminergic neurons, and (2) if dopaminergic neurons increase activity in response to blue light or if they are downregulated to promote sleep and thus recovery from oxidative damage. This will help determine whether the oxidative stress caused by blue light is sufficient to affect dopaminergic neuronal function and thus lead to neurodegenerative ailments.

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Poster 21: Evaluation of sleep and circadian traits as risk factors for substance use disorders

Nicole Fairbanks
University of Pittsburgh

N Fairbanks1, C Forbes1,2, S Stringfield1,2, A Sved1,2, J Zeak1, M Seney1,2, Y Huang1,2, M Torregrossa1,2, C McClung1,2

Adolescence is a period of substantial neurodevelopment and behavioral changes, increasing their vulnerability to drug use and developing substance use disorders. Developmental changes in sleep and circadian rhythms, primarily represented by a shift towards a more evening chronotype, are increasingly recognized as relevant to understanding adolescent addiction vulnerability. Thus, this natural variation in sleep and circadian traits seen in adolescence may be associated with indicators of risk for substance use, such as altered cognitive function, and impulsivity. To investigate this interaction, male and female adolescent heterogenous stock rats were screened on measures of sleep duration and circadian chronotype prior to being either tested on measures of impulsivity, attention and cognition using the 5-choice serial reaction time task (5-CSRTT), and for propensity to self-administer nicotine, or underwent electrophysiology recordings of Nucleus Accumbens (NAc) action potential firing. Our results showed that circadian period had the strongest relationship with impulsivity. Specifically, rats with a short circadian period were more impulsive and made more premature responses. We also found that circadian period was strongly associated with measures of nicotine self-administration, where a longer circadian period was associated with more total nicotine intake and greater lever pressing. These results suggest that natural variation in sleep and circadian traits, like having a short or long circadian period, may be associated with different risk factors of substance use disorders.

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Funding Support: National Institute on Drug Abuse (USA), P50DA046346

Poster 23: Meta analytical behavioral metrics to enhance motor phenotype reliability

Zoe Bichler
The Jackson Laboratory

VD Knickerbocker1, T Laster1, J Osgood1, K Perron1, N Stroud1, J Suckovic1, C Wise2, JM Wotton2, and Z Bichler1

C57BL/6J mice represent the predominant inbred background in preclinical research, with extensive phenotypic characterization available through resources such as the Mouse Phenome Database (https://phenome.jax.org/). Despite this breadth, inter-laboratory variability in experimental design, protocol implementation, and under-powered designs frequently limit the reliability and reproducibility of reported phenotypes. At the same time, many additional mouse strains are routinely used, further highlighting the need for harmonized reference data.

To address this, we leveraged the large, continuously expanding dataset generated at The Jackson Laboratory's Neurobehavioral Phenotyping Core since 2019, comprising mice tested under harmonized protocols, controlled environmental parameters, and validated operator procedures. Using aggregated multi-assay datasets, we derived strain-specific and age-stratified reference ranges for key motor and activity-related phenotypes. Analytical efforts included composite score generation, inter-assay correlation matrices, and cross-modal concordance analyses to evaluate redundancy and discriminative sensitivity across widely used motor assays.

Our initial objective was to develop recommendations for aging studies using C57BL/6J as a benchmark strain. We identified assays most sensitive to age- and sex-related differences, estimated empirically supported group-size thresholds, and examined correlations across assays to highlight complementary or redundant motor measures. These analyses provide a framework for selecting reliable phenotypes and designing efficient behavioral pipelines.

Ultimately, we hope this effort will contribute to improved reproducibility, more strategic and ethical study design, and broader alignment with the 3Rs (Replacement, Reduction, Refinement) principles in *in vivo* behavioral research.

1Neurobehavioral Phenotyping Core at the Center for Biometric Analysis, 2Center for Biometric Analysis, The Jackson Laboratory, Bar Harbor, Maine, USA.

Acknowledgement: The authors would like to extend the co-authorship to all former staff

members of the Neurobehavioral Phenotyping core at the Center for Biometric Analysis at The Jackson Laboratory as they have generated or help generate essential data needed for this work.

Poster 25: Characterizing chronic alcohol withdrawal-induced pain: cold hypersensitivity and neuronal hyperexcitability across two models of alcohol dependence

James DeMarsh
University of Pittsburgh

JW DeMarsh[1,6], AJ Brandner[1,6], GE Homanics[3,4], BK Taylor[2,3,6], SP Farris[2,5,6]

Alcohol Use Disorder (AUD) is a chronic, relapsing condition in which pain during withdrawal- collectively termed chronic alcohol withdrawal-induced pain (CAWIP)- contributes to continued alcohol use and relapse. Despite its clinical relevance, the behavioral and neurophysiological mechanisms underlying CAWIP remain poorly understood. This study broadly characterizes CAWIP using complementary behavioral and electrophysiological measures across two mouse models of chronic alcohol exposure. Adult C57BL/6J mice underwent either chronic intermittent ethanol vapor (CIEV) exposure to model dependence or a novel one-bottle access (1BA) voluntary drinking paradigm as a comparison model. Pain-related behaviors were assessed via von Frey testing for cold hypersensitivity, and conditioned place pereference/aversion (CPP/CPA) for spontaneous pain-like behaviors. To investigate underlying neurophysiological changes, excitatory neurons in the parabrachial nucleus were examined for markers of hyperexcitability, spontaneous activity, and membrane resistance. Withdrawal from both CIEV and 1BA exposure produced cold hypersensitivity and mechanical hypersensitivity alongside electrophysiological recordings that confirmed increased neuronal hyperexcitability, spontaneous activity, and altered membrane resistance. Together, these findings suggest that CAWIP involves intrinsic central neuronal changes that may drive sensory hypersensitivity during withdrawal. These results advance our understanding of the multidimensional nature of CAWIP and set the stage for future studies to uncover the central neuronal mechanisms driving this phenomenon and discover more clinically-relevant therapeutic targets.

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Poster 27: Drosophila metabotropic glutamate receptor homologs mangetout and mGluR differentials affect locomotion in adult-specific neuronal knockdown

Mandi Luellen
California State University, Fresno

Mandi Luellen, Melody S. Kirby, and Cynthia T. Hsu

Metabotropic glutamate receptors (mGluRs) are G-protein-coupled receptors that regulate neurotransmission, synaptic plasticity, and neuronal excitation. Disruptions to mGluR function have been implicated in psychiatric disorders including schizophrenia and mood disorders. Here, we present preliminary data characterizing knockdown of two metabotropic glutamate receptor homologs in Drosophila, mangetout (mtt) and metabotropic glutamate receptor (mGluR), in sleep, locomotor, and feeding assays. We find that constitutive pan-neuronal knockdown of mangetout is lethal while adult-specific knockdown leads to drastic impairments in locomotion. In contrast, while previous studies have reported sleep deficits in constitutive pan-neuronal knockdown of mGluR, we find that adult-specific knockdown does not compromise sleep or locomotion. This suggests that the role of mGluR in sleep is developmental, in contrast to mtt, which has significant effects in adult behavior. Supplementing mtt

knockdown flies with octopamine and serotonin precursors does not rescue locomotor phenotypes. Our findings may indicate an overlooked role of mtt as a homolog for metabotropic glutamate receptors and a potential target for pharmacological screens.

California State University, Fresno

Poster 29: Identifying activity and ciradian patterns in marmosets (Callithrix jacchus) with genetic risk for Alzheimer's disease

Abbey Setlik
University of Pittsburgh

Abigail Setlik1, Rishabh Choudhary1, Lauren Bailey1, Takeshi Murai1, Lauren Mongeau1, Emily Rothwell2, Afonso Silva2, Stacey J. Sukoff Rizzo1,2

Emerging data indicate that impairments in motor function and alterations in sleep may precede Alzheimer's disease (AD) pathology and related cognitive decline by several years. The present studies investigated early changes in activity and circadian patterns in common marmosets *(Callithrix jacchus)* with genetic mutations in the PSEN1 gene which confers early onset AD in humans. Nanowatches (Camntech LTD) were attached to the collars of freely moving male (n=16) and female (n=22) marmosets (aged 1-6 yrs). Individuals were evaluated longitudinally over up to a 3-year period which revealed behavioral changes with aging and disease progression measure by AD related biomarkers. Ongoing studies continue to investigate the earliest behavioral changes associated with genetic risk for AD as a functional prodromal biomarker to reveal the earliest activity changes that precede disease and cognitive decline.

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Poster 31: Utilizing Zebrafish to Understand How ap2s1 Regulates Habituation Learning

Adore Ferguson-Richards
Haverford College

Adore D. Ferguson-Richards, Justin Minerva, Jacob A. Krawitz, Nicky Rashkover, Roshan A. Jain

To adapt to our environment, our nervous system is constantly interpreting sensory information allowing us to react to relevant stimuli while ignoring the irrelevant ones. Which allows for the appropriate response to the stimuli based on the current environmental constraints. One of the main contributors to this is a form of non-associative learning called habituation, characterized by a decline in responsiveness to a stimulus after repeated exposure. Habituation learning is evolutionarily conserved across all animals from invertebrates up to humans. Habituation learning can vary in a number of neuropsychiatric conditions in humans including Attention-Deficit/Hyperactivity Disorder (ADHD) and Autism Spectrum Disorder (ASD). There are multiple genes associated with these conditions; the one we chose to focus on is AP2S1. This gene encodes a subunit of the AP2 complex which assists in clathrin mediated endocytosis however its role in behavioral regulation is not well understood. When ap2s1 is mutated in zebrafish, larvae show deficits in habituation of the acoustically-evoked escape response, making this a useful model to understand the mechanism of this learning. The fast escape response of five day old larval zebrafish is controlled by a pair of command neurons called the Mauthner cells. When a Mauthner cell fires, larvae perform a fast escape, so we reason that habituation requires regulating these critical neurons. The Spiral Fiber neurons are excitatory neurons that are presynaptic to the Mauthner cells and are thought to regulate Mauthner firing. We investigated the activity of the Spiral Fiber termini, which bundle together around the start of the Mauthner axon as well as the Spiral Fiber cell bodies. We used a fluorescent calcium indicator (GCaMP5G) to visualize Spiral fiber structure and activity to determine if there is an association between the development of these neuronal structures and the altered habituation of the mutants. For the wild type siblings there is activity at the Spiral Fiber termini which reduces as the fish habituate. In the mutants there is higher activity observed at the Spiral Fiber termini compared to the wild type. To understand why I am measuring the dimensions of the bundle of Spiral Fiber termini as well as counting the cell bodies to determine if there's a developmental difference between the mutants and the siblings. By characterizing the circuit development of Spiral Fiber neurons we hope to better understand how ap2s1 regulates habituation learning.

Poster 33: VIP Signaling in the Prefrontal Cortex Promotes Alcohol Motivation and Reshapes Cortical Output Circuits

Dakota Brockway
University of Pittsburgh

Dakota F. Brockway, Samuel L. Boehm, Nilah D. Jordan, Max E. Joffe

Alcohol use disorder is characterized by maladaptive decision making and persistent alcohol seeking, yet the circuit mechanisms within the prefrontal cortex that drive alcohol motivation remain poorly understood. Vasoactive intestinal peptide (VIP) interneurons are powerful regulators of cortical network activity through disinhibitory circuit motifs and VIP neuromodulatory signaling. However, the role of VIP interneurons in alcohol-related behaviors has not been established. We investigated how VIP signaling in the prelimbic prefrontal cortex regulates alcohol motivation and prefrontal circuit activity. Whole-cell patch clamp electrophysiology revealed that VIP directly depolarizes VIP interneurons, suggesting that VIP signaling can amplify VIP interneuron activity within cortical circuits. Consistent with this mechanism, intoxicating doses of systemic alcohol administration increased calcium activity in VIP neurons measured using fiber photometry in vivo. To determine whether VIP neuron activity contributes to alcohol seeking, we

manipulated VIP neurons during operant alcohol self-administration. Chemogenetic activation of VIP neurons significantly increased motivation for alcohol under progressive ratio schedules, indicating that VIP neuron activity promotes alcohol-seeking behavior. In addition to regulating interneuron activity, VIP signaling differentially modulated layer 5 pyramidal neurons depending on projection subtype. Electrophysiological recordings revealed that VIP excitation enhanced excitability in extratelencephalic neurons while suppressing action potential firing in intratelencephalic neurons, suggesting that VIP signaling may bias prefrontal output toward specific downstream circuits. Together, these findings identify VIP interneurons as a previously unrecognized regulator of alcohol motivation and suggest that alcohol recruits VIP signaling to reshape prefrontal cortical output.

Poster 35: Metals exposure impairs flight performance in Drosophila melanogaster

Justine Anne Guevarra
University of Maryland, Baltimore County

Justine Anne A. Guevarra[1] and Fernando Vonhoff[1]

Alzheimer's Disease (AD) is the most common form of dementia and is associated with mutations in certain genes including APP. The human APP gene encodes for amyloid precursor protein (*App*), which is a transmembrane protein expressed in several tissues and organs, including the brain. Although its physiological functions remain elusive, studies in mice and fruit flies show that lack of *App* proteins results in developmental and locomotor deficits, which can be rescued by the expression of the human *App*, confirming its conserved properties. *App* is also a metalloprotein, which can bind to metals such as copper and zinc. Interestingly, recent studies display increasing evidence on the possible link between metals and neurodegenerative disorders. Post-mortem AD brains reveal accumulation of metals such as aluminum, copper, zinc, etc. To our knowledge, there is no known study investigating the possible protective role of the fly ortholog, *appl*, on metal exposure in flies. This project aims to investigate the effects of metal exposure in flight behavior in *Drosophila melanogaster*. Current observations showed a decline in flight performance in *appl* null flies compared to wild-type flies following aluminum treatment, suggesting a protective role for *appl*. Surprisingly, copper treatment did not influence the *appl* null flies but resulted in lower survival rate and flight performance in wild-type flies. Overall, the knowledge gained from the experiments will serve as a reference for future studies in mammals and broaden the understanding of the mechanisms involving *App* / *Appl* and metal toxicity in flies.

[1]Department of Biological Sciences, University of Maryland, Baltimore County, USA

Poster 37: Gustation discrimination task for detecting Alzheimer's related pathologies in aging marmosets (Callithrix jacchus).

Lauren Mongeau
University of Pittsburgh - Aging Institute

Lauren R. Mongeau[1], Lauren Bailey[1], Takeshi Murai[1], Abbey Setlik[1], Andrew DeSana[1], Afonso Silva[2], Stacey J. Sukoff Rizzo[1,2]

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Introduction: Alzheimer's disease (AD) is a neurodegenerative disorder and the most common form of dementia. While a primary symptom is cognitive decline, sensory deficits including impairments in taste and smell have emerged as early indicators of amyloid and tau pathology that precede cognitive impairment. The ability to discriminate the functional changes related to variation in normal healthy aging from those that lead to pathological aging, and the onset of AD may help predict the earliest accumulation of AD pathology and enable better detection, earlier diagnosis, and interventions for prevention and treatment. For these studies we leveraged a large population of marmosets including marmosets seeded with tau to evaluate the trajectory and progression of AD pathology and sensory and cognitive impairments.

Methods: Male and female marmosets across an age span ranging from 18 months to 16 years utilized for this task. Marmosets were initially habituated to the presentation of a panel fixed with two drinking bottles placed on the front of their homecages that both contained a 20% solution of marshmallow juice (MJ) formulated from dehydrated marshmallows into drinking water for a 10-minute session. Following the habituation session in which we verified that marmosets sampled from both bottles, we conducted a two-choice preference test in subsequent sessions with varying solutions of MJ (0.5%, 1%, 5%, 10%) or water, randomized for testing order and counterbalanced for side using a modified latin-square design. All 4 sessions were conducted on consecutive days of the week with a second week of testing presenting the same testing order but the opposite presentation for side from the previous week. The bottles were weighed before and after the session to determine the amount consumed and the preference ratio of the water to MJ. A dummy bottle was placed on the opposite side of the panel to account for potential leakage.

Results: Preference ratio was evaluated of animals that have tau pathology. All Marmosets could discriminate against the higher concentrations of 10% and 5% MJ relative to water. We saw differential responses at the 1% and 5%,

Conclusion: With this, we can establish typical marmoset taste discrimination longitudinally in wild-type marmosets, and marmosets with higher risk of tau pathology. Analysis of these studies provide characterization of gustation discrimination and decline in healthy marmosets and marmosets with risk of tau pathology across lifespan, which are being correlated with aging and AD related biomarkers to inform and predict cognitive decline associated with non-specific dementia.[LM1]

*add funding information here

- [LM1]Dementia progression (non-specific) impairs taste and smell (Waldton, 1974; Lang et al., 2006 Steinbach et al., 2010)

Poster 39: Sex-Specific Genetic Influences on Creativity: COMT Polymorphisms and Divergent Thinking in Elite Judo Athletes

Sigal Ben-Zaken

Levinsky-Wingate Academic College

Shani Beicher (Raphael) and Sigal Ben-Zaken, PhD

Creativity—the capacity to generate novel and valuable solutions—represents a complex cognitive phenotype with substantial inter-individual variation. While heritability estimates from twin studies suggest 40-70% genetic contribution to creative abilities, the molecular genetic architecture underlying creativity remains poorly understood. The dopaminergic system, particularly genes regulating dopamine metabolism and signaling in the prefrontal cortex, has emerged as a promising candidate pathway given dopamine's established role in cognitive flexibility and executive function.

Motor creativity, defined as the ability to generate diverse and novel motor solutions to environmental challenges, represents a specialized form of creative cognition critical for performance in dynamic, unpredictable contexts such as combat sports. Elite Judo—a combat sport requiring rapid tactical decision-making and execution of complex motor sequences under pressure—provides an ideal model system for investigating the genetic basis of motor creativity. Furthermore, Judo offers unique advantages for studying sex differences, as it is one of few combat sports with gender parity in competitive participation and achievement.

Despite extensive research on cognitive creativity, motor creativity has received minimal attention, and potential sex-specific genetic influences have been virtually unexplored. Understanding these mechanisms has broad implications for behavioral genetics, extending from talent identification in specialized domains to personalized cognitive enhancement strategies.

Methods

We conducted a genetic association study in 77 elite Israeli Judo athletes (32 females, 45 males; aged 16-25 years) representing national and international competitive levels. Participants completed validated assessments of divergent thinking (verbal and figural creativity measuring fluency, flexibility, and originality) and sport-specific motor creativity tasks. Buccal epithelial samples were genotyped for three functionally relevant polymorphisms in the dopaminergic system: *COMT* A/G rs4680 (catechol-O-methyltransferase, regulating prefrontal dopamine catabolism), *BDNF* C/T rs6265 (brain-derived neurotrophic factor, modulating dopamine neuron survival), and *DRD2* C/T rs6277 (dopamine receptor D2, affecting receptor expression and signaling).

Results

Female athletes exhibited significantly higher scores across all creativity dimensions compared to males (fluency, flexibility, and originality; all $p < 0.05$). Moderate to high correlations emerged between divergent thinking measures and motor creativity scores ($r = 0.45$-0.68), particularly in males, suggesting partially shared cognitive mechanisms.

Critically, we identified a significant sex-by-genotype interaction for *COMT* rs4680 across all figural divergent thinking dimensions ($p < 0.05$). Female athletes carrying the GG genotype (low COMT activity, higher prefrontal dopamine) demonstrated the highest creativity scores, while male athletes with the AA genotype (high COMT activity, lower prefrontal dopamine) showed superior performance. This interaction remained significant after controlling for training history and competitive achievement level. No significant main effects of genotype or interactions were observed for *BDNF* rs6265 or *DRD2* rs6277 polymorphisms.

Discussion

Our findings reveal sex-specific genetic influences on creative cognition, with opposite effects of the *COMT* rs4680 polymorphism in males versus females. This pattern suggests that sex hormones modulate the relationship between prefrontal dopamine tone and creative thinking. Estrogen and testosterone receptors are abundantly expressed in prefrontal cortex and interact with dopaminergic signaling pathways. The observed interaction may reflect sex-differential optimal dopamine levels for creative cognition, consistent with inverted-U models of prefrontal dopamine function.

These results extend beyond athletic performance, providing insight into fundamental mechanisms of sex differences in cognition and the complex interplay between genetic variation, sex hormones, and neurotransmitter systems in shaping behavioral phenotypes. Future research should examine additional dopaminergic polymorphisms, investigate the neural mechanisms mediating these sex-specific effects using neuroimaging approaches, and determine whether similar gene-by-sex interactions influence creativity in non-athletic populations and other cognitive domains.

Poster 41: Social Stress Engages BNST Kappa Opioid Receptors to Escalate Alcohol Consumption

Praneetha Panthagani

Louisiana State University Health Sciences Center

F. Paliarin, E. Doré, P. Panthagani, S. Mirza, L. Finlay, T. Nguyen, E. Weiser, C. Duplantis, and R. Maiya

Social stress is a critical driver of escalated alcohol use and relapse; however, the associated molecular mechanisms are poorly understood, limiting the identification and evaluation of therapeutic targets. We have demonstrated using Social Defeat Stress (SDS) model that alcohol consumption was escalated in

both male and female C57BL/6J mice. Stress related behaviors have been shown to be mediated via Dynorphin/Kappa opioid receptor (Dyn/KOR) system. When administered systemically with a long-acting KOR antagonist like Norbinaltorphimine (NorBNI), both male and female stress animals reduced alcohol consumption, with minimal effect on unstressed controls. Stress escalated alcohol consumption was ameliorated in Oprk1-Cre male mice after chemogenetic activation of KOR expressing neurons in Basolateral Amygdala (BLA^{KOR}). Both KOR antagonism and chemogenetic activation of BLA^{KOR} terminals in Bed Nucleus of Stria Terminalis (BNST) attenuated escalation of alcohol consumption in both males and females, implicating the BLA^{KOR}-BNST pathway in SDS induced drinking. In line with these findings, we found that, KOR deletion in the BLA diminished SDS-escalated alcohol consumption in female mice. We further observed increased prodynorphine (pDyn) expression in Dorsal raphe nucleus (DRN) and enhanced activation of BNST-projecting DRN^{Dyn} neurons following social stress, suggesting these neurons as a key source of Dyn recruited by SDS. Future experiments will examine the causal role of DRN^{Dyn} neurons in SDS-induced alcohol escalation following deletion of KOR in BLA-BNST pathway.

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Funding: R01AA031733, R01AA027293 and LSUHSC startup funds.

Poster 43: Clocking the effects of sleep loss: the neurogenetic intersection of sleep disturbance and disordered behaviour in Drosophila

Abigail Bechard
University of Western Ontario

A.T. Bechard[1], R. Ataei[1], & A.F. Simon[1]

Sleep disturbance is strongly connected to increases in disordered behaviour such as inattention, social avoidance, and hyperactivity across species. There is increasing evidence that the bidirectional relationship between disturbed sleep and disordered behaviour may be modulated by changes in gene expression. Mutations in genetic regulators of dopamine availability and circadian rhythm have been associated to this relationship, but how expression of such genes may mediate these effects has yet to be explored. To investigate this connection, flies (*Drosophila melanogaster*) are a great model due to their genetic tractability and thoroughly characterized behavioural assays. In this study, the effect of sleep disturbance on daily locomotor activity and sleep are measured in flies with altered expression of primary circadian regulator *period* (*per*) or *dopamine transporter* (*dat*). Preliminary results indicate that *dat* mutants of both sexes display an exaggerated locomotor response to the mechanical stimulation used to disrupt sleep. Also, *dat* may play a more prominent role in female sleep compared to males, and *per* a stronger role in males. In addition to gene expression analysis via RT-qPCR, next steps include behavioural assays on *dat* and *per* mutants to measure the effect of sleep disruption on hyperactive, inattentive, and social behaviours. The results of this study will help to establish the symptomatological relationship between sleep disturbance and the expression of genes regulating circadian rhythm and dopamine availability.

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Funding support: internal grants to AFS and ATB; NSERC Fellowships to ATB; NSERC Discovery grants 05054-2022 to AFS.

Date: Thursday, 11/June/2026

7:30am - 8:00am	**Registration** Location: **Foyer at Assembly Room entrance**
8:00am - 10:00am	**Selected Talks 2** Location: **Assembly Room/Ballroom** Session Chair: **Gregg Homanics** Session Chair: **Carlos Novoa** Session Chair: **Aijun Zhang** Session Chair: **Antonio Marini-Davis**

CaMPARI2 enables stimulus-locked whole-brain activity mapping at cellular resolution in unrestrained larval zebrafish

Roshan Jain
Haverford College

KR Robbins1, A Bredbenner1, RA Osbaldeston2, KS Villafañe2, EE Shin2, E Merkulova1, A Clevenger1, PB Delean2, C Campos2, GC Peet2, RA Jain1,2

Visualizing active neurons and circuits *in vivo* is critical for investigating the neural activity that underlies behavior. While several established methodologies are available to achieve this end in larval zebrafish, they are limited by the scale of tissue visualization, temporal resolution, need to restrain larvae, and/or accessibility of necessary instruments. Here, we establish a pipeline for the visualization and quantification of spatiotemporally precise whole-brain neural activity in larval zebrafish using CaMPARI2, a genetically encoded photoconvertible calcium indicator. Using temporally specific photoconverting UV light exposures, we capture whole-brain “snapshots” of neural activity time-locked to stimuli during unrestrained larval behavior. We optimized experimental conditions for establishing sub-second neuronal activity changes across acoustically-evoked behavioral paradigms spanning minutes to hours. We then leveraged this system to pinpoint brain-wide neural activity changes during nonassociative habituation learning, observing distinct activity signatures in the subpallium, preoptic area, and habenulae that are altered through pharmacological and/or genetic disruption of habituation learning. This approach effectively complements the temporal precision achievable through post hoc activity detection methods and expands the accessibility of large-scale behavioral circuit dissection beyond highly specialized real-time volumetric imaging equipment.

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Funding Support: NIH R15EY031539

Molecular signatures of maladaptive plasticity in the amygdala in a rat model of chronic neuropathic pain

Igor Ponomarev
Texas Tech University

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Neuropathic pain is a chronic pain condition that results from damage or dysfunction in the nervous system. While mechanisms of neuropathic pain at the peripheral and spinal cord level have been extensively studied, pain mechanisms in the brain remain underexplored. The amygdala, a limbic brain region, has emerged as a critical brain area for the emotional-affective dimension of pain and pain modulation. Amygdala neuroplasticity has been associated with pain states, but exact molecular and cellular mechanisms underlying these states and the transition from acute to chronic pain are not well understood. Here, we used the spinal nerve ligation model of neuropathic pain in male rats to investigate changes in gene expression in two amygdala nuclei, basolateral (BLA) and central (CeA) at the chronic pain stage using RNA sequencing. We used an integrative approach that focuses on functional significance and cell type specificity of differentially expressed genes to nominate mechanistic targets for central regulation of chronic pain. Our integrative transcriptomic and bioinformatic analyses identified individual genes (e.g., *Cxcl10, Cxcl12, Mbp, Plp1, Mag, Mog, Slc17a6, Gad1, Sst*), molecular pathways (e.g., cytokine-mediated signaling pathway), biological processes (e.g., myelination, synaptic transmission), and specific cell types (e.g., oligodendrocytes, glutamatergic and GABAergic neurons) affected by chronic pain. Our results also provide evidence for hemispheric lateralization of pain processing in the amygdala. Overall, our study proposes oligodendrocyte dysfunction in the amygdala, neuroimmune signaling in the CeA, and glutamatergic neurotransmission in the BLA as mechanistic determinants of and potential therapeutic targets for the management of chronic neuropathic pain.

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Funding Support: National Institutes of Health grants R01 NS038261 to V.N. and I.P. and R01 AA027096 to I.P.

Central Amygdala Ninein Deletion Alters Ethanol Anxiolysis, Consumption, and GABAergic Function

Michael Miles
Virginia Commonwealth University

Emma Gnatowski[1,2], Jessikah Buys[1,2], Jensen Goulette[2,3], Andrew A. George[1] and Michael F. Miles[1,2]

Acute ethanol reduces anxiety in humans and animal models. Anxiety disorders increase risk for Alcohol Use Disorder (AUD) and human subjects report that stress and anxiety increase ethanol consumption. The Miles laboratory previously identified the microtubule binding protein Ninein (Nin) as a candidate gene underlying ethanol's acute anxiolytic-like properties in BXD recombinant inbred mice. Here we report on behavioral, gene expression and GABAergic function consequences of Nin deletion in central amygdala (CeA). Deletion of Nin in CeA was done using stereotactic injections of AAV8-hSyn-GFP (control) or AAV8-hSyn-CRE-GFP (deletion) virus in Ninfl/fl mice. CeA Nin deletion increased acute ethanol anxiolysis in the light-dark box assay in male and female mice and reduced intermittent access 2-bottle choice ethanol consumption and preference x 5 weeks in female but not male mice. There were no changes in ethanol sedation (loss-of-righting reflex) or pharmacokinetics. Taste preference for quinine or saccharin were also unaffected. Bulk RNAseq analysis of stereotactic injection sites in CeA revealed striking evidence of neuroinflammatory and GABAergic gene expression alterations in Nin deletion mice. Preliminary electrophysiological studies on CeA IPSP activity measured by voltage clamp analysis showed Nin deletion altered IPSC duration, suggesting a post-synaptic site of action. **Conclusions:** These studies document that Nin function in CeA modulates the acute anxiolytic and consumption properties of ethanol, with the latter showing a striking sex preference. Initial mechanistic studies suggest that disruption of Nin expression in CeA produces changes in post-synaptic GABA receptor function, with coincident gene expression changes consistent with altered GABAergic neuron homeostasis and possible synaptic remodeling.

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Long Noncoding RNA Gas5 Modulation of the Stress-Related Phenotypes of Chronic Intermittent Ethanol Vapor Exposure

Rachel Rice
University of Pittsburgh

RC Rice,[1] MN Wauhop,[2] GE Homanics,[3,4] SP Farris[2,5]

The long noncoding RNA *growth arrest specific 5* (*Gas5*) is differentially methylated in blood and brain of individuals with alcohol use disorder and has multiple proposed functions, including immune- and glucocorticoid signaling modulation. In mouse, we previously observed a male-specific increase in nondependent voluntary ethanol consumption following *Gas5* knockdown (KD) in medial prefrontal cortex (mPFC) and persistent downregulation of mPFC *Gas5* following chronic intermittent ethanol vapor (CIEV). We hypothesize mPFC *Gas5* modulates CIEV-induced escalation of ethanol consumption and stress-related behaviors during ethanol abstinence. To test this, we performed mPFC-specific *Gas5* KD in adult male mice (C57BL/6J background), exposed them to CIEV-2BC, then tested stress-related behaviors and peak serum CORT levels during abstinence. mPFC *Gas5* KD did not alter voluntary ethanol consumption, but modulated CIEV effects on stress-related behaviors during abstinence. In the open field test, *Gas5* KD in air controls mimicked the stress-like phenotype of CIEV, measured by decreased entries and time spent in the inner zone, whereas *Gas5* KD attenuated this phenotype in CIEV mice. In the elevated plus maze, CIEV resulted in decreased stretch-attend postures, which was ablated in *Gas5* KD CIEV mice, suggesting *Gas5* KD reversed CIEV impairment on risk assessment behavior. Peak serum CORT increased in CIEV mice seven days into abstinence, with no effect of *Gas5* KD. However, 21 days into abstinence, CIEV mice with *Gas5* KD displayed a persistent increase in serum CORT, while their CIEV control counterparts displayed levels resembling air controls. These findings suggest *Gas5* modulates stress-related phenotypes of ethanol abstinence.

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Funding Support: NIAAA AA031168 (PI: Rice), U01 AA020889 (PI: Farris, MPI: Homanics), and R01 AA024836 (PI: Farris).

Dimensional mapping of mouse behavior reveals clusters enriched for neuropsychiatric disorder related phenotypes

Markos Michail Chatzigiannis
Fujita Health University

Markos Michail Chatzigiannis1,2, Hirotaka Shoji2 , Daiki Sato2,3,4 , Keizo Takao, Tsuyoshi Miyakawa2

Behavioral phenotyping across genetically modified mouse strains is extensive but lacks a coherent framework for cross strain comparison. We assembled a large scale dataset comprising more than 10,000 mice from 167 strains across 15 behavioral assays. Multifactor analysis identified two principal dimensions, locomotor activity and learning/memory, that captured the dominant components of cross strain covariance. Clustering along these axes defined six behavioral phenotypes reflecting systematic variation in activity and cognitive performance. To assess clinical relevance, each strain was assigned a disorder association score derived independently of mouse behavioral data from publicly available human gene–disease association resources. Scores were calculated for intellectual disability (ID), autism spectrum disorder (ASD), schizophrenia, and major depressive disorder. Disorder association differed across endotypes, with the strongest and most consistent enrichment observed for ID and ASD. Strains with high ID or ASD burden were concentrated in the same two profiles characterized by comparable learning impairments but opposite locomotor patterns: one predominantly hypoactive and the other hyperactive. Across disorders, specific behavioral indices showed selective correlation with disorder burden, identifying the most informative measures for distinguishing disorder relevant models. These results indicate that clinically distinct diagnostic categories share underlying behavioral structure in mouse models that is not captured by disorder titles alone. This framework enables the interpretation of large scale behavioral data and the evaluation of disorder relevance for genetically modified mice.

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Core circadian clock neurons regulate activity of insulin-producing cells

Annika Barber

Rutgers University

Naureen Hameed[1], Sergio L. Crespo Flores[1], Evan Cirone[1], Chenyue Zhao[1], Annika F. Barber[1,2,*]

Central pacemaker neurons use a combination of external stimuli and neuropeptide signaling to synchronize molecular oscillations leading to circadian behaviors. The clock network structure and signaling between these pacemaker neuron groups have been well described, but how these pacemakers communicate with specific brain output regions remains poorly understood. Here, we identified how “core” clock neurons in *Drosophila*, the ventrolateral neurons (LNvs), signal to the proto-hypothalamic region, the *pars intercerebralis* (PI). Previously thought to communicate with the PI only indirectly, we provide evidence to show that LNvs functionally modulate, the PI’s insulin-producing cells (IPCs) in a time-of-day-dependent manner. This functional connectivity relies on neuropeptidergic signaling of two canonical clock neuropeptides: pigment dispersing factor (PDF) and short Neuropeptide F (sNPF). Loss of either receptor alone in PI subpopulations does not alter feeding or locomotor rhythmicity. Further, we provide insight into how these two neuropeptides may be acting together via their receptors to signal to IPCs. We identify sexually dimorphic responses of IPC response to LNv stimulation, which may be partially explained by sex differences in proximity of clock neurons to the PI. Our findings indicate that LNvs form both direct peptidergic signaling but also form indirect multisynaptic circuits with IPCs, which may model more broadly how they communicate with various other clock output regions.

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10:00am - 10:30am

Break
Location: **Assembly Room**

10:30am - 12:30pm

Symposium 5: Bridging the Rodent to HumanTranlational Gap: Marmosets as Model Systems for the Study of Alzheimer's Disease

Location: **Assembly Room/Ballroom**
Session Chair: **Lauren Bailey**
Session Chair: **Stacey Rizzo**

Generation of genetically engineered marmosets with AD risk mutations

Gregg Homanics

University of Pittsburgh

GE Homanics[1], SJ Sukoff Rizzo[2], AC Silva[2], PL Strick[2], GW Carter[3], JE Park[2]

Mutations in the presenilin 1 (*PSEN1*) gene are the most common cause of familial, early-onset Alzheimer's disease (AD), yet rodent models fail to fully recapitulate human AD pathology because they do not naturally develop amyloid plaques or tau aggregates. The common marmoset (*Callithrix jacchus*) offers a compelling alternative. It is a small non-human primate whose brain closely resembles the human brain, and it develops spontaneous age-related amyloid and tau pathology. We used CRISPR/Cas9 gene editing to independently introduce two *PSEN1* point mutations (C410Y and A426P) — the same single-nucleotide changes found in human patients — into marmoset embryos. Several genetically engineered founders were produced; however, most died prematurely. One C410Y founder survived to adulthood and sired germline offspring. Phenotypic outcomes observed in these animals will be the subject of companion talks. In addition, experiments are underway to develop marmoset models of sporadic, late-onset AD.

These nonhuman primate models are unprecedented for studying the earliest molecular events that initiate AD, evaluating preventive interventions, and bridging the rodent-to-human translational gap.

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Behavioral characterization of marmosets with genetic risk for AD

Lauren Bailey
University of Pittsburgh

Lauren Bailey, PhD[1], Takeshi Murai, PhD[1], Lauren Mongeau[1], Abbey Setlik[1], Tingting Zhang, PhD[1], Seung-Kwon Ha, PhD, DVM[1], Gregory W. Carter[2], Afonso C Silva, PhD[1] and Stacey J Sukoff Rizzo, PhD[1],

Fundamental questions remain regarding the mechanisms that initiate Alzheimer's disease (AD), drive its progression, and link pathology to cognitive impairment. As part of our MARMO-AD consortium, we established a comprehensive testing battery sensitive to detecting age-dependent cognitive decline across the lifespan in our colony of aging marmosets, and marmosets genetically engineered with mutations in the PSEN1 gene which confers early onset AD in humans. Beginning in adolescence, marmosets are trained using touchscreens through a battery of tests that captures a spectrum of cognitive domains including spatial working memory (delayed match to position), behavioral flexibility and reversal learning (delayed non-match to position), recognition memory (trial unique delayed match to sample), attention (serial reaction time task), and episodic-like memory (paired associative learning task). Behavioral and cognitive function are aligned with longitudinal PET neuroimaging and blood-based biomarkers to track AD progression. Similar to human PSEN1 mutation carriers, plasma Aβ42:40 is significantly elevated relative to non-carrier controls Despite robust biomarker and pathological changes, PSEN1 marmosets show no significant deficits in task acquisition or performance across cognitive domains up to 4 years of age. These data are not surprising and recapitulate the disease trajectory of increased amyloid in plasma and brain years before cognitive decline. These ongoing longitudinal studies are enabling the identification of the molecular and cellular mechanisms other than amyloid that contribute to and precede cognitive decline associated with Alzheimer's disease progression.

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This work is supported by U19AG074866 and 5T32AG021885-19.

From Marmosets to Man: Building a Translational Platform to Advance Alzheimer's Disease Research

Amantha Thathiah
University of Pittsburgh School of Medicine

Thais Rafael Guimarães[1], Jung Eun Park[1], Catrina Spruce[2], Stephanie Hachem[1], Swati Banerjee[1], Lauren K Hayrynen Schaeffer[1], Gregg E Homanics[1], Stacey J Sukoff Rizzo[1], Gregory W Carter,[2], Afonso C Silva[1], and Amantha Thathiah[1]

Progress in preclinical Alzheimer's disease (AD) research has been constrained by models that fail to faithfully recapitulate human aging and overt AD neuropathology. The common marmoset (*Callithrix jacchus*), a New World non-human primate, exhibits aging trajectories, genetic heterogeneity, and complex social behaviors closely resembling those of humans, providing a highly translational platform for age-related neurodegenerative research. Importantly, marmoset studies uniquely enable longitudinal correlation of *in vitro* cellular models with *in vivo* assessments across the marmoset lifespan. We performed an integrated *ex vivo* and *in vitro* characterization of marmoset AD and tauopathy models. Immunohistochemical analyses of postmortem brains revealed robust amyloid-β (Aβ) and tau pathology, along with the associated cellular pathology. To establish an *in vitro* cellular system, we adapted a well-established human direct reprogramming protocol to generate age-conserved induced neurons (iNs) from marmoset fibroblasts. Comparative, unbiased RNA-seq analyses of marmoset and human iN conversion trajectories revealed significant species-specific differences, guiding targeted optimization of the reprogramming strategy. The refined protocol achieved high-efficiency neuronal conversion, improved cell survival and maturation, and preserved AD-relevant protein expression, including amyloid precursor protein (APP)/Aβ and tau. Together, this integrated framework establishes the marmoset as a powerful translational model for AD research. This platform enables minimally invasive mechanistic studies, longitudinal analyses, high-throughput drug screening, and therapeutic discovery aimed at accelerating disease-modifying strategies for AD.

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Comparative genetics of AD: from mice to marmosets to humans

Greg Carter
The Jackson Laboratory
Gregory W. Carter

Laboratory mice and marmosets provide potential models of aging and Alzheimer's disease with the capacity to track the initiation and progress of pathology across compressed lifespans. We have performed genetic, genomics, and proteomic analyses of plasma and postmortem tissues to map multi-modal aspects of disease development and progression in these model species. We have drawn from human genetic studies to engineer multiple mouse strains carrying candidate genetic factors for late-onset Alzheimer's disease. In the marmoset, we are combining genetic engineering of select loci with outbred genetic variability to analyze biomarkers of aging and dementia. We assembled a new telomere-to-telomere reference genome for the common marmoset (Callithrix jacchus) and performed whole-genome Illumina sequencing on over 230 marmosets. For both model systems, we have used high-coverage proteomics, Alamar NULISA, and transcriptomics to assess the disease-relevant consequences of genetic factors. We have identified a broad range of disease-associated signatures in knock-in mouse and marmoset models, including immune, metabolic, and synaptic alterations. These outcomes frequently correlate with clinically relevant biomarkers and behavioral outcomes. We have also used multi-omic signatures to understand the molecular pharmacodynamics of candidate drugs. Our findings constitute a data and model resource for identifying the appropriate animal model for understanding genetic liability in Alzheimer's and preclinical testing of targeted therapeutics.

The Jackson Laboratory, Bar Harbor, ME, USA

12:30pm - 1:30pm	**Lunch** Location: **Lower Lounge/Ballroom**
1:30pm - 2:30pm	**IBANGS Business Meeting**
2:30pm - 4:00pm	**Plenary 4: Distinguished Investigator Award Lecture (Professor Leslie Griffith)** Location: **Assembly Room/Ballroom** Session Chair: **Karla Kaun**
6:00pm	**Closing Banquet** Location: **The Porch Restaurant**

Location, location, location

Leslie Griffith
Brandeis University

Leslie C. Griffith MD PhD

Neurons are among the most structurally and functionally specialized cells in the body, capable of processing, integrating, and transmitting information. Unlike many other cell types, neurons exhibit extreme morphological polarity, with distinct compartments—dendrites, soma, axon, and synaptic terminals—each requiring specifically tailored protein populations to support their localized functions. The recognition that cells are capable of locally synthesizing proteins marked a major shift in our thinking about cellular organization. Local translation enables neurons to establish functionally distinct subdomains and it allows rapid, site-specific responses to activity, supporting structural changes that are required for long-term plasticity. Localized protein synthesis and compartment-specific protein turnover allow neurons to dynamically respond to activity and environmental changes.

I will talk today about the first plasticity-related protein to be shown to be locally synthesized in neurons: Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII). Activity-dependent CaMKII synthesis is conserved across phyla and occurs in both pre- and postsynaptic compartments. Activity also has an additional effect on CaMKII that is equally conserved: it causes a subcellular redistribution of the protein. How the dynamic regulation of CaMKII levels and subcellular localization are related is not understood, and I will discuss recent results from my lab that address these questions. My talk will highlight how the unique biology of neurons depends on highly specialized protein landscapes—proving that in neuroscience, just like real estate, success ultimately comes down to location, location, location.

Nancy Lurie Marks Professor of Neuroscience, Brandeis University