Friday May 13, 2016

Selected Talks 1: Featured Speaker

To drink or Not(ch) to drink: conserved neuro-molecular mechanisms underlying alcohol cravings

Michael Feyder, Emily Petruccelli, Rachel Muster, Nicolas Ledru, Kristin Scaplen, Karla R Kaun

Many highly conserved molecular mechanisms underlying pattern formation, neuronal development and circuit formation are also important for neuronal function in the adult brain. Notch signaling, for example, plays a critical role in neurogenesis and neuronal differentiation, but is also required in adult neurons for synaptic and structural plasticity. Notch therefore influences behaviors such as memory and sleep both through its effects on neuronal development, and on neuronal function in the adult. We propose that Notch signaling is one of the main contributors underlying the remarkably conserved behavioral effects of alcohol intoxication. Our work in Drosophila provides direct evidence that alcohol induces immediate changes in Notch signaling that lead to transcriptional changes required for neuronal plasticity. Moreover, these changes occur in circuit motifs required for memory formation that are shared between flies and mammals. This work thus reveals a highly conserved mechanism that clarifies how alcohol cravings differ from other forms of appetitive memories, and suggests that one of the keys to cracking circuit function is to investigate the adult roles of the molecules required for circuit development.

Selected Talks 1: Speaker 2

Machine learning to identify highly heritable components of substance use disorders

Jinbo Bi[1], Jiangwen Sun[1], Henry R Kranzler[2]

Substance use disorder (SUD) is clinically heterogeneous and a SUD diagnosis is based on multiple clinical criteria. Identifying highly heritable components or subtypes of SUD could maximize the likelihood of finding genetic associations. Existing methods for refinement of disease phenotypes perform unsupervised cluster analysis on clinical criteria and hence do not assess heritability. Existing heritable component analytics either cannot utilize general pedigrees or have to estimate a large covariance matrix of clinical features from limited samples, which leads to inaccurate estimates and is often computationally prohibitive. These methods are also difficult to correct the fixed effects from covariates such as age or sex to identify truly heritable components. The proposed approach searches for a combination of clinical features and directly maximizes the heritability of this combined trait. A quadratic optimization problem is derived where the objective function is formulated by decomposing the maximum likelihood method for heritability estimation. This new approach is

computationally efficient and generates linearly-combined traits of higher heritability than those by other methods, with correction for fixed effects. Using 6,810 subjects with 13 cocaine use and related behavioral variables, this method yielded a subtype of cocaine use disorder (CUD) with early first-use of cocaine, early onset of dependence, heavy cocaine use, and an estimated heritability of 0.7. A genome-wide association study, that compared the utility of the derived subtype with the commonly used CUD phenotype, identified more statistically significant associations with the derived subtype with replication.

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Selected Talks 1: Speaker 3

Genomic responses in mouse models greatly mimic human inflammatory / psychiatric diseases.

Tsuyoshi Miyakawa[1], Keizo Takao[2], Hideo Hagihara[1]

The use of mice as animal models has long been considered essential in modern biomedical research, but the role of mouse models in research was challenged by a recent report that genomic responses in mouse models poorly mimic human inflammatory diseases (Seok et al., PNAS, 2013). We have been investigating the molecular basis of psychiatric disorders using gene expression analyses in mice models of the disorders. The analysis methods include a statistical test, “Running Fisher”, which considers fold change rank of gene expressions in a non-parametric way and is a very sensitive informatics method to detect similarity between two different sets of gene expression data. By applying the same analysis methods we have been using, we reevaluated the same gene expression datasets used in the study by Seok et al. Contrary to the previous findings, the gene expression levels in the mouse models showed extraordinarily significant correlations with those of the human conditions (Spearman's rank correlation coefficient: 0.43-0.68; genes changed in the same direction: 77-93%; P = 6.5 × 10(-11) to 1.2 × 10(-35)). Moreover, meta-analysis of those datasets revealed a number of pathways/biogroups commonly regulated by multiple conditions in humans and mice. These findings demonstrate that gene expression patterns in mouse models closely recapitulate those in human inflammatory conditions and strongly argue for the utility of mice as animal models of human disorders.

We will introduce the debates ignited by the studies and also discuss the general significance of mice models of human diseases.

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Selected Talks 1: Speaker 4

EEG source imaging indices of cognitive control show association with dopamine system genes

G McLoughlin[1], J Palmer[2], S Makeig[2], N Bigdely-Shamlo[2], T Banaschewski[3], M Laucht[3, 4] & D Brandeis[3,5,6,7]

Cognitive control is critical for higher mental abilities and among the most heritable of neurocognitive traits. Two candidate genes, catechol-O-methyltransferase (COMT) and DRD4, which both have roles in the regulation of cortical dopamine, have been consistently associated with cognitive control. We predicted that the variation of the availability of dopamine in the prefrontal cortex related to the different polymorphisms would differentially affect measures of cognitive flexibility and cognitive stability. We precisely examined the cortical indices of cognitive control at the source level via independent component analysis (ICA) and dipole projections in a healthy population sample of 174 participants. The homozygous Val allele of the COMT genotype was associated with an increase in the source-projected P3 ERP elicited by inhibitory stimuli compared to those with the Met allele. Furthermore, an interaction effect emerged, which indicated abnormal cortical responses to response execution in those with the 7-repeat allele only if they have the homozygous Val/Val polymorphism or the homozygous Met/Met polymorphism but not in those with the heterozygous Val/Met polymorphism. Our findings correspond with previous evidence that too little or too much prefrontal dopamine is disruptive and impairs cognitive control functioning. Our finding that the Met/Met polymorphism is associated with impaired response inhibition performance shows that while the increased dopamine in frontal cortical areas associated with this polymorphism has advantages for some tasks, there may be a compromise in response inhibition functioning. The improved functional and anatomic separation of the cortical sources of EEG gives measures that may better elucidate the complex mechanisms behind genetic effects on brain function.

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Selected Talks 1: Speaker 5

COMT Allelic Variation and Sleep Organization in Human Neonatal Opioid Withdrawal

K.M. Daigle[2], M.J. Hayes[1, 2], H. Shrestha[3], B.A. Logan[4], N.A. Heller[5], M.S. Brown[6], D.A. Nielsen[7], & E.M. Wachman[3]

Neonatal Abstinence Syndrome (NAS) is a common complication of prenatal exposure to opioids such as methadone. NAS severity is associated with poor neonatal sleep. Work from our group (Wachman et al., 2013) has shown that withdrawal severity in NAS differs based on neonatal variants in the COMT (catechol-O-methyltransferase) gene, such that minor allele carriers showed shorter hospitalization and reduced pharmacotherapy. The rs4680 SNP in the COMT gene encodes a valine to methionine substitution at amino acid 158 (Val158Met). COMT encoded by the valine allele catabolizes dopamine approximately four times faster and more efficiently than does the methionine allele. It was proposed that COMT genotypes would predict neonatal sleep organization in the early post-birth period. Sleep studies were conducted in prenatally methadone-exposed (n=35) and nonexposed (n=20) neonates from 2400-0500 h on PND 1 or 2 using videosomnography and actigraphy, and compared with genetic data obtained from saliva samples (Genotek OGR-250 kit with CS-1). Using TaqMan technology, COMT rs4680 (assay C_25746809_50) was analyzed using a dominant model (AA vs. AG/GG genotypes). Patient status was compared to sleep-related variables (e.g., sleep state, arousal, and movement parameters) using a two-factor GLM with maternal prenatal alcohol consumption as a covariate. Preliminary results for gene-behavior corollaries revealed that neonates with AG/GG genotypes had comparatively more robust spontaneous movements, less arousal and cry, and increased sleep frequency and duration compared to AA genotype neonates (all p's<.05). The AG/GG genotype, found to be associated with a milder NAS phenotype, also is correlated with improved neonatal sleep.

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Selected Talks 1: Speaker 6

Amygdala-cortical interaction reveals underlying connectivity mechanisms in 5-HTTLPR genetic polymorphism variation

Qian Luo[1,2], Tom Holroyd[3], Derek Mitchell[4], Henry Yu[2], Xi Cheng[5], Colin Hodgkinson[6], Daniel McCaffrey[2], David Goldman[6], R. James Blair[2]

Carriers of the short alleles (S-carriers) of the Serotonin Transporter Gene (5-HTTLPR) show an elevated amygdala response to emotional stimuli relative to carriers of the long allele (LL-homozygous). However, it is unknown whether this reflects increased responsiveness of the amygdala generally or interactions between the amygdala and the specific input systems. It is argued that the amygdala receives input via a quick subcortical and a slower cortical pathway. If the elevated amygdala response in S-carriers reflects generally increased amygdala responding, then group differences in amygdala should be seen across the time course of the amygdala response. However, if the difference is a secondary consequence of, for example, enhanced amygdala-cortical interaction, then group differences might only be present later in the amygdala response. Using MEG and advanced source imaging techniques providing fine-scale spatiotemporal information, we recruited healthy volunteers and compared S-carriers and LL-homozygotes viewing fearful, angry and neutral expressions. We found an enhanced amygdala response to fearful relative to neutral expressions from 40-50ms after stimulus onset. However, group differences in this amygdala response were only seen 190-200ms after stimulus onset. These group differences were preceded by increased STS responses in S-carriers from 130-140 ms after stimulus onset. An enhanced amygdala response to angry relative to neutral expressions was noticed from 260-270ms after stimulus onset. These group differences were preceded by increased STS responses in S-carriers from 150-160 ms after stimulus onset. These data suggest that previous reports of enhanced amygdala responses in S-carriers might reflect enhanced STS-amygdala connectivity in S-carriers.

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Outstanding Travel Award Talks

The Bone Morphogenetic Protein (BMP) Signaling Pathway is Required for Allodynia in Drosophila melanogaster

K Gjelsvik[1], M Galko[2], G Ganter[1]

According to the NIH, more than 100 million people suffer from chronic pain in the United States, yet the mechanism of this pain sensitivity is not well understood. The ultimate goal of this research is to develop drug targets that can block the activity of candidate molecules, thus blocking the sensitized pain state and thereby alleviating chronic pain. A candidate gene approach was used to identify novel components required for the modulation of the pain sensitization pathway in Drosophila melanogaster. Several components of the Bone Morphogenetic Protein (BMP) signaling pathway were investigated using an RNAi knock-down approach. A UV-induced model was used to damage the larval epidermis, creating a sensitized pain state. This was followed by behaviorally assaying the larvae with a normally non-noxious thermal stimulus. The response latencies of mutants and controls were recorded and compared, thereby testing for allodynia, or a response to a normally non-noxious stimulus. The results of this study found that the knockdown of various BMP components significantly decreased the formation of sensitization compared to the controls, indicating that those components are necessary for the formation of allodynia.

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Endocannabinoid Signaling as a Modifier of Zebrafish Stress Responses
Randall G. Krug II[1,2], Morgan O. Petersen[3], and Karl J. Clark, Ph.D. [1,2,3]

The number of annual cannabis users exceeds 100,000,000 globally and an estimated 9% of these individuals will suffer from dependency, but a dearth of knowledge exists about the potential consequences on public health. However, the psychoactive constituents of cannabis are known to affect the endocannabinoid (eCB) system, and disrupt features of vertebrate physiology and behavior. Our central hypothesis is that disruptions in the eCB signaling system have pathological consequences on vertebrate behavior and physiology, including dysregulation of the stress response system. Herein, we use a preclinical zebrafish model to clarify the ramifications of disturbances in the eCB signaling system. Using qRT-PCR and *in situ* hybridization we show that the genes encoding enzymes that synthesize (*abhd4, gde1, napepld*), enzymes that degrade (*faah, faah2a, faah2b*), and receptors that bind (*cnr1, cnr2, gpr55-like*) eCBs are expressed throughout development. We show that disruptions of this system via exogenous cannabinoid administration results in altered behavior and physiology, including increased secretion of glucocorticoids in our stress response reporter line. We are developing a zebrafish eCB signaling mutant library using TALENs and show that disruption of *faah2a* alters stress-associated behavior. Collectively, these results establish zebrafish as a viable model for studying eCB signaling, and lay a foundation for informing a better understanding of the toxicological and therapeutic potential of the eCB system.

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Support: DA032194, Mayo Graduate School, and Mayo Foundation.

Identifying novel therapeutic epigenetic targets for the treatment of bipolar disorder

RW Logan[1], AR Ozburn[2,3], X Zhu[1], E Fitzgerald[1], RN Arey[4], MB Jarpe[5], C Wang[6], and CA McClung[1]

Altered epigenetic and circadian mechanisms are implicated as putative contributors to the pathophysiology of mood and addiction disorders, including bipolar disorder (BD). Circadian genes that comprise the transcriptional—translational feedback loops of the molecular clock regulate neurophysiology underlying 'emotionality'. Molecular rhythms are modulated by epigenetic enzymes called histone deacetylases (HDACs), which are capable of inducing stable transcriptional changes. Both molecular rhythms and HDAC activity are targets of first line medications for BD, including valproic acid (VPA). VPA specifically inhibits class I/IIa HDACs, although whether HDAC inhibition underlies the therapeutic effects are unclear. We have previously demonstrated *Clock*Δ19 mutant mice display a behavioral repertoire with high validity to human bipolar mania (e.g., circadian and sleep disruptions, hyperactivity, reduced anxiety and depression, hyperhedonia, among others). The present study investigated whether VPA and more specific HDAC inhibitors are capable of 'normalizing' anxiety and depression-like behaviors of *Clock*Δ19 mice. We found chronic treatment with VPA, SAHA (class I/II), and MS275 (class I), but not MC1568 (class IIa), normalized the anxiety and depression-like behaviors of both male and female mutant mice. Novel compounds that specifically inhibit the class I HDACs, HDAC1 and/or HDAC2 also normalize these mania-like behaviors. AAV-shRNA mediated knockdown of *Hdac2,* but not *Hdac1,* in the brains of mutant mice produced similar behavioral effects. Studies are currently underway using RNA-seq in mouse and human iPSC models to elucidate the transcriptional networks underlying these potentially 'therapeutic' effects of class I HDAC inhibition, and more specifically, targeted inhibition of HDAC2 for the treatment of BD.

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KCC2 and Peripartum stress hyporeactivity: Implications for Vulnerability to Postpartum Depression

LC Melón[1], JL Maguire[1]

The early postpartum period is characterized by dampened reactivity of the hypothalamic-pituitary adrenal (HPA) axis. Previous work from our lab demonstrated that, in virgin mice, activation of hypothalamic CRH neurons involves neurosteroid-dependent regulation of KCC2, the potassium chloride co-transporter, and a subsequent reduction in integrity of GABAergic inhibition. Given significant changes in neurosteroidogenesis associated with pregnancy, we aimed to determine whether stress hyporeactivity during peripartum involved disrupted regulation of KCC2. For this, we used the Cre/lox system to generate CRH-specific KCC2 knockout mice (CRH-KCC2$^{-/-}$) and compared their virgin and postpartum stress and anxiety-like behavior to wild-type controls. Given the relationship between stress regulation and the development of postpartum depression, we assessed depressive-like and maternal approach behaviors in these mice. Western blots of hypothalamic lysates confirmed that the stress-associated regulation of KCC2 is disrupted for postpartum mice. ELISAs also confirmed blunted corticosterone release for these females. Both of these postpartum associated changes were not seen in CRH-KCC2$^{-/-}$ dams. CRH-KCC2$^{-/-}$ dams failed to develop the postpartum-associated reduction in anxiety-like behavior in the elevated plus maze and light/dark test seen in WT mice. Although depressive-like behavior in the Porsolt forced swim test was not conclusively shown for CRH-KCC2$^{-/-}$ dams, these females showed unique degradation of their maternal behaviors following exposure to forced-swim stress. These data support a role for KCC2 regulation of CRH neurons in the expression of peripartum stress hyporeactivity. Further, they suggest a role for stress hyporeactivity in the resilience of maternal behaviors and mood during the postpartum period.

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Support: NINDS-R01NS073574 (JLM) and NIGMS-K12GM074869 (LCM)

Symposium 1: The evolving role of corticotropin releasing factor (CRF) system in physiology and behavior

Interoceptive Nutrient Sensing by the Brain

Greg S. B. Suh

Sugars in the natural environment can be detected through taste-dependent and taste-independent modalities. Taste-dependent modalities consist mainly of

peripheral chemosensory neurons such as sweet taste receptors, which primarily detect the orosensory value of sugar (i.e. sweetness)[1]. Evidence of a taste-independent modality - a post-ingestive sugar sensor - that detects the nutritional value of sugar has been shown in insects[2-4] and mammals[5,6]. However, the identity of the post-ingestive sugar sensor and the mechanism by which animals respond to the nutritional content of sugar independently of orosensory value is unknown. My laboratory identified six neurosecretory cells in the *Drosophila* brain that produce Diuretic hormone 44 (Dh44), a homologue of the mammalian corticotropin-releasing hormone (CRH), were activated by nutritive sugars and not by nonnutritive sugars. Flies in which the activity of these neurons or the expression of the *Dh44* gene was disrupted failed to select nutritive sugars over nonnutritive ones after periods of starvation. Notably, artificial activation of Dh44 receptor-1 neurons dramatically increased the rate of proboscis extension reflex (PER) responses, promoting food intake. This manipulation also resulted in frequent episodes of gut contraction and excretion. Together, we propose that the Dh44 system directs the detection, ingestion, and digestion of nutritive sugar through a positive feedback loop to continue consumption of nutritive sugar.

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The Role of CRH in the Human Stress Response and in Melancholic and Atypical Depression

Philip W. Gold, M.D.[1]

Shortly after Vale and his colleagues isolated and sequenced CRH, they demonstrated that its intracerebroventricular administration to the rat set into motion the full repertoire of behavioral and physiological responses characteristic of the stress response. These included fear-related behaviors, anxiety, hyperarousal, decreased appetite, decreased sleep, hypercortisolism, and

inhibition of the endocrine programs for growth and reproduction. Melancholic depression is associated with anxiety (often directed at the self and experienced as a sense of worthlessness), hyperarousal, loss of appetite, early morning awakening, activation of the hypothalamic-pituitary axis, and inhibition of the growth and reproductive axes. Thus, melancholic depression resembles a stress response that has become hyperactive and excessively prolonged. Antidepressants preferentially effective in melancholia consistently down-regulate hypothalamic CRH gene expression. iIn contrast, atypical depression seems the antithesis of melancholia, and is characterized by lethargy, fatigue, and both excessive eating and sleep. Atypical depression, thus, represents a stress response that has become hypoactive. We have developed a CRH receptor antagonist, antalarmin, that, is orally absorbed and that readily crosses the blood-brain-barrier. We have shown that in rhesus macaques under severe stress, antalarmin blocks the appearance of anxiety, increases sexual behavior, and attenuates the responses of plasma ACTH, cortisol, norepinephrine, epinephrine, and of CSF CRH. Utilizing antalarmin, we have also shown that CRH exerts multiple proinflammatory effects in the periphery. This consists of CRH in the periphery degranulating mast cells or released from sensory nerve endings to promote adverse dermatologic phenomena. CRH also plays a prominent role in autoimmune phenomena.

[1] Intramural Research Program, NIH/NIMH

Unraveling the connectivity- function relationship of corticotropin releasing factor (CRF) neurons employing zebrafish

Mahendra Wagle and Su Guo

In 1948, Harris proposed that hypothalamic neurons produce discrete factors that regulate the release of pituitary hormones. A search lasting over three decades has led to the discovery of corticotropin releasing factor (CRF), the principal stress hormone of the brain. CRF not only drives the HPA axis, but also coordinates many humoral and behavioral aspects of the adaptive response to stress. Despite these significant advances, it remains not well understood how CRF neurons carry out their distinct functions at the circuit level. A common population of CRF neurons may regulate both physiological and behavioral responses to stress, or alternatively, distinct subpopulations of CRF neurons may be dedicated to regulating physiological or behavioral responses. In this study, we address the circuit mechanism of CRF neuronal function in larval zebrafish, a vertebrate genetic model organism that is amenable to cellular and molecular dissection of circuit function. The function of subsets of CRF neurons in regulating both behavioral and physiological responses to stress will be elucidated at single-cell resolution. This work will potentially shed important light on the fundamental mechanisms underlying neuronal connectivity and function, and unveil suitable assays for discovering genes and small molecules that regulate stress reactivity in vivo.

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Functional interplay between CRHR1 and endocannabinoid signaling in the regulation of stress and anxiety

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Abstract: Corticotropin releasing hormone (CRH) and endocannabinoids have opposing influences on how the brain responds to stress. The CRHR1 receptor acts throughout the brain/pituitary to increase endocrine and behavioral responses to stress, while the cannabinoid type 1 receptor (CB1) suppresses these outputs. Although these systems have some overlapping distribution, few studies have investigated if these systems interact. Our studies reveal a novel mechanism of CRHR1 regulation of hydrolysis of the endocannabinoid anandamide (AEA) through an induction of fatty acid amide hydrolase (FAAH). Specifically, activation of CRHR1 in the amygdala enhances FAAH activity and suppresses AEA signaling. More so, CRHR1 activity, specifically on glutamatergic neurons in the basolateral nucleus of the amygdala (BLA), mediates stress-induced FAAH activity. Consistent with this, the majority of CRHR1 positive cells in the BLA co-express FAAH, and local inhibition of FAAH within the BLA is capable of attenuating CRH-induced anxiety and activation of the HPA axis. Consistent with this role of CRH-mediated regulation of endocannabinoid signaling, CRH-overexpressing mice exhibit constiuitive upregulation of FAAH activity and reductions in AEA content within the amygdala. Additionally, the ability of sustained glucocorticoid exposure to suppress AEA signaling is mediated by the upregulation of extra-hypothalamic CRH. Finally, a potential role of this CRH-FAAH/AEA interaction to contribute to trait anxiety was revealed by selective breeding studies in rats. Together, these data demonstrate that induction of FAAH activity by CRH contributes to the generation of anxiety and emotionally aversive states in response to stress.

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Poster Session 1 (Odd Numbers)

1. Sex differences in the behaviour of the 3xTg-AD mouse model of Alzheimer's Disease

Richard E. Brown[1], Kurt Stover[1], Kyle Roddick[1], and Leanne Stevens[1]

We examined age-related changes in spatial learning and memory, procedural learning and memory, and olfactory memory in male and female 3x-Tg AD

mouse models of AD and their wildtype (WT) controls between 3 and 18 months of age. The 3xTg-AD mouse has the human amyloid precursor protein (APPswe), a mutated mouse presenilin-1 (PS1M1461), and a transgene associated with tau pathology (Tau301L). The B6129S1F2 mice are used as controls. The data presented examines genotype, age and sex differences in multiple memory; visuo-spatial, motor and olfactory learning and memory. We also examine sex differences in longevity, frailty and neuropathology. We found genotype, and sex differences in a number of learning and memory tasks. Sex differences were found in both WT and 3xTg-AD mice, but there were a number of sex differences in the 3xTg-AD mice that were not found in WT mice, indicating the importance of analyzing sex differences in transgenic mice.

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Support: NSERC of Canada.

3. Profiling differential expression of miRNA in the prefrontal cortex of tobacco smokers vs. non-smokers.
MS Powers[1], X Gallego, & MA Ehringer[1]

Small non-coding RNAs have gained a lot of attention in recent years for their role in post-transcriptional regulation of gene expression and their potential role as biomarkers of disease states and treatment responses. Of the non-coding RNAs, the ~22 nucleotide microRNAs (miRNAs) show utility as potential biomarkers of disease. A number of disease states show altered miRNA expression profiles including cancer, liver disease and psychiatric conditions like drug addiction. Along with their utility as biomarkers, profiling differentially expressed miRNAs between case and control populations has the potential to direct attention to novel mechanistic pathways underlying the disease being investigated. The goal of this study was to sequence and profile mature miRNA expression in prefrontal cortices of postmortem human brain samples received from the National Development and Research Institutes (NDRI). Using *Illumina Small RNA-Seq*, we profiled miRNA collected from 10 brain samples (5 smokers, 5 non-smokers) and analyzed differentially expressed miRNAs using the Partek suite of tools (Bowtie Aligner, Expectation/Maximization quantification algorithm and an ANOVA differential expression comparison). Seven mature miRNAs were differentially expressed between smokers and non-smokers with $p < 0.05$ (adjusted for multiple comparisons); 2 miRNAs were up regulated in smokers, and 5 were down regulated. These are the first data to suggest differential expression of miRNA in human brain tissue samples from smokers and suggest that nicotine use can change miRNA expression profiles in the human brain.

[1]Institute for Behavioral Genetics, University of Colorado Boulder; Boulder, Colorado, USA. Funding **Support:** P60 DA011015, R01 AA017889, and T32 AA007464

5. How universal is the 'Epigenetic Clock'?

Louis Y. El Khoury[1], Tyler J. Gorrie-Stone[1], Jonathan Mill[2], Leonard C. Schalkwyk[1]

Background: A widely cited epigenetic DNA methylation Clock model (Horvath, 2013) is useful at determining the age of unknown samples across a wide breadth of human tissues. It has received further attention as a tool for assessing the rate of aging in a given tissue or disease state. This is a tremendously helpful concept, but the assumption that this is a universal clock has limits. We look at brain and blood tissues from elderly subjects, who are not heavily represented in the 2013 study.

Results: Age predictions using the model are younger than the chronological age for all tissues tested: prefrontal cortex (13.94 ± 6.65 years, n=84), enthorhinal cortex (10.25 ± 6.71 years, n=79), superior temporal gyrus (12.48 ± 5.95 years, n=87), cerebellum (26.71 ± 7.52 years, n=83), and blood (8.90 ± 5.81 years, n=57).

Discussion: Plots of predicted vs chronological ages show an inflection around retirement age, with a decreased slope in old age, indicating that for some of the loci included in the model, change with age slows or stops. This effect was previously reported in cerebellum, and is seen most strongly in this tissue, but is also present in three regions of the cortex and in the blood. The Horvath(2013) model uses a custom transform of the data to treat young subjects differently, and thus already has an inflection at the age of 20. We show that at least with the current coefficients, it should have another one at approximately 65.

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7. Exploring Intersections in Mental Health and Addiction: A Screen for Psychiatric Drug Efficacy in Nicotine Cessation

J Yang[1], CL Daby[2], C Grudzinski[3], MA Cousin[4], RM Harm[5], SC Ekker[1,2], EW Klee[4]

Tobacco use is the leading cause of preventable mortality worldwide, resulting in approximately six million deaths annually. Although gains have been made to decrease morbidity and mortality associated with tobacco, its use remains a disproportionate burden for patients with mental illnesses. A 2013 CDC study found that 36% of Americans with mental illness smoke cigarettes, accounting for a disparate ~30% of all cigarettes smoked by U.S. adults. Given the overlap between neural pathways implicated in broader mental health and substance abuse, comorbid pathophysiology is predicted to exist. Here we utilize the zebrafish model system as a moderate throughput screen to identify drugs with potential efficacy in nicotine cessation. This project specifically concentrates on FDA-approved pharmacotherapies utilized in the treatment of mental health disorders. Larval zebrafish are treated overnight with each candidate drug and then subjected to a nicotine locomotion assay. Efficacious candidates attenuate the nicotine locomotor response without attenuating cinnamon or mustard oil

control response. An external collaborator will evaluate potential candidates using conditioned place preference assays. The screen currently has 49 psychiatric drugs in various states of completion. Four drugs exhibit nicotine-specific attenuation, and seven additional drugs require further control testing. Analysis of efficacious candidates may identify overlapping mechanisms involved in mental illness and substance abuse and perhaps drive future pharmacotherapies available for mental health patients who wish to quit tobacco.

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Support: National Science Foundation Graduate Research Fellowship Program (USA), NIH-NCATS CTSA Grant Number UL1 TR000135, CEBRA

9. Involvement of long-term and transgenerational hippocampal Akt-mTOR signaling impairment in behavioral deficiency in the F1 and F2 generations of postpartum depression-like mice

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Postpartum depression (PPD) increases vulnerability of the offspring to psychiatric disorders including depression. Akt-mTOR signaling in the hippocampus is implicated in depression but its role in the behavioral deficits in the offspring of PPD remained unknown. Here, by using a prepregnancy stress model of PPD in which females with experience of prepregnancy chronic stress showed long-lasting postpartum depression-like behaviors, we tested depression-like behaviors in the PPD offspring (F1) at juvenile and adulthood. The male F1 was mated with a normal female to produce F2. Hippocampal Akt-mTOR signaling was examined in the F1 and F2 generations of PPD, and mice treated with the rapid antidepressant ketamine. PPD-F1 showed depression-like behavior at juvenile and adulthood, evidenced by reduced sucrose preference (SP), longer immobility time in the forced swim test (FST), and longer latency to feed and less food consumed in the novelty suppressed feeding (NSF) test. PPD-F1 showed AKT-mTOR signaling deficiency in the hippocampus, with the down-regulated expression of p-AKT, p-mTOR and p-p70S6K. A single dose of ketamine reversed the behavior deficiency and the impairment in expression of AKT-mTOR signaling of PPD-F1. Furthermore, the F2 mice still demonstrated deficits in the SP and NSF tests and hippocampal AKT-mTOR signaling, with normalized performance in FST. The present study using a novel PPD model for the first time demonstrated the long-term and transgenerational effects of PPD on the behavior of offspring. Our finding also suggests that the impaired Akt-mTOR signaling may at least partially contribute to the depression–like behaviors across generations.

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Support: Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD); Natural Science Foundation of Jiangsu Province (Grant No. BK20151568)

11. A comparative phenotypic analysis of nicotine effects and dependence in C57BL/6J and C57BL/6N mouse strains

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Mouse substrains can be a powerful source for discovery of genes and pathways regulating complex behavior. In this study, we report that C57BL/6J (B6J) and C57BL/6NCrl (B6N) substrains, differ significantly in nicotine pharmacology and dependence after acute and chronic administration. We characterized behavioral and pharmacological responses to nicotine male adult B6J and B6N mice in a battery of tests. We measured nicotine's acute effects (antinociception and hypothermia), repeated (locomotor sensitization), conditioned place preference (CPP) as well as withdrawal signs after chronic exposure to the drug. In general, B6N mice were less sensitive than B6J mice to nicotine's acute effects and nicotine CPP. In contrast, we found that B6N has a higher sensitized locomotor response to nicotine than B6J. While both B6N and B6J expressed physical and affective withdrawal signs in nicotine-dependent mice, withdrawal signs were more intense in B6N mice. In addition, nicotine metabolism and levels did not differ between the two substrains after acute and chronic administration. We are currently testing whether the *Cyfip2* (S968F) mutation that is known to regulate psychostimulant response could also contribute to the differences seen in nicotine response. Together, these results provide a thorough, simultaneous evaluation of the pharmacological and behavioral differences to experimenter-administered nicotine as measured in several behavioral tests of aspects that contribute to smoking behavior. These results suggest that these substrains may be useful for future genetic studies on nicotine behaviors.

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Support: This research was supported by the National Institute on Drug Abuse R01DA032933.

13. Resources for translational behavioral, neurological and addiction research at the Rat Genome Database

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RGD (http://rgd.mcw.edu), the premier online resource for rat genetic, genomic and phenotypic data, offers a large body of cross-species functional, phenotype and disease data and multiple innovative software tools, facilitating the search for appropriate models for human diseases including neurological disorders such as multiple sclerosis, and behavioral conditions such as alcoholism and anxiety disorders. RGD's gene records feature diverse functional annotations including gene ontology, disease, phenotype, pathway and gene-chemical interactions, as well as links to orthologs for ready access to species-specific information. RGD annotates QTL for rat and human, as well as importing MGI's QTL data and phenotype annotations, linking genomic regions to diseases, phenotypes and traits. Rat strain data includes Mammalian Phenotype and disease annotations, as well as specific quantitative phenotype data. This data, available through the PhenoMiner tool, details a specific value for each measurement as well as specifying the method used and the experimental conditions under which the measurement was made. In this way, researchers can compare within or across studies to determine the effect of a specific condition or to view differences between strains for the same measurement and conditions. RGD's strain-specific variant data (available on gene report pages, through the Variant Visualizer or via the RGD FTP site) allows researchers to search for sequence variations which might be associated with their observed phenotypes. Interesting variants in rat can also be compared to clinical variants in human imported from NCBI's ClinVar database via RGD's gene ortholog data.

[1]Rat Genome Database, Medical College of Wisconsin, Milwaukee, WI, USA
Support: NHLBI R01 HL064541

15. The genetics of phenotypic tradeoffs between stress-resistance and cognition

Alexis Hill[1] and Y Ben-Shahar[1,2]

Organismal resistance to neuronal stress favors reduced neuronal plasticity. In contrast, adaptive cognition relies on increased neuronal plasticity. Consequently, it seems that neuronal processes that support a robust homeostatic stress response would carry a cognitive cost and *vice-versa,* a highly evolved cognition would lead to a reduced resistance to stress. However, whether intrinsic neuronal constrains can lead to phenotypic tradeoffs between the homeostatic stress response and cognition has not been investigated. Studies in the Ben-Shahar lab investigate this fundamental question with genetic, neurophysiological, cellular, and behavioral approaches. To achieve our goals, we use the power of *Drosophila* genetics to identify genome-level architectures, and specific molecular mechanisms, that drive and maintain neuronal and behavioral homeostasis, and the possible mechanistic constrains between

neuronal homeostasis and plasticity. To date, our data indicate that the regulation of expression of specific voltage-gated potassium channels play a major role in the intrinsic capacity of neurons to maintain excitability at a homeostatic set-point, and the ability of neurons, and behaving animals, to adjust to rapid changes in the environment.

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Support: NIH R21NS089834, NSF 1322783, and The McDonnell Center for Cellular and Molecular Neurobiology

17. Identifying genes associated with conditioned fear in the Diversity Outbred mouse population using a forward genetic, genome-wide approach

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Traditional forward genetic studies in mice have struggled with insufficient recombination and have generally failed to identify causal genes. This has led to increased interest in the development of genetically diverse, highly recombinant mouse populations, allowing for a greater phenotypic variation and improved mapping resolution. We tested 509 male JAX Diversity Outbred mice (DO) using a three-day conditioned fear (CF) paradigm to fine-map quantitative trait loci (QTLs) associated with acquisition, extinction, and renewal of CF. A one-way repeated measures ANOVA found a significant increase in freezing following each tone-shock pairing during acquisition, ($F_{1.8, 892.8} = 799.503$, $p < 0.0001$; $\eta_p^2 = 0.612$), demonstrating the ability to learn to associate the tone and foot-shock. Freezing behavior in response to the tone significantly decreased across trial-blocks during extinction training ($F_{5.9, 2973.2} = 177.986$, $p < 0.0001$; $\eta_p^2 = 0.260$) suggesting mice were able to successfully extinguish the fearful association over time. On the renewal test, mice displayed less freezing relative to the first trial-block of extinction training ($t(506) = 28.561$, $p < 0.0001$). QTL analyses on a subset of mice (N = 288) identified numerous suggestive and significant QTLs associated with conditioned fear on chromosomes 1, 6, 7, 9, 11, 12, 16, and 18. As we increase our sample size, mapping power and resolution will improve. Additionally, we have collected hippocampal tissue from a subset of mice for future RNA-Seq experiments that will enable us to explore the network of correlations between CF, genetic variation, and expressed genes.

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19. An e-internship program in Neuroscience for K12 students

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There is a wealth of Neuroscience related data available in open access repositories along with free web tools for data exploration and analysis. It is well established that mining and assessing these data can aid research efforts. Learning Biology in high school typically involves memorizing vast amounts of descriptive material without much context being provided. Consequently, most students turn off to Biology without ever experiencing the rewarding aspects that we, as researchers, experience daily. Through the e-internship program, we engage and help train students by offering an inquiry-motivated learning opportunity in authentic research using Bioinformatics. Communication is done via the Internet using an open-source learning platform, video conferencing, email, and document sharing. The start point for the projects involves the identification of brain regions associated with a behavioral process or brain disease (using fMRI data especially). Subsequently, gene expression data for the relevant brain regions are analyzed to uncover patterns, correlations, and networks. From this experience, students gain a foundation for a prospective career and transferable skills and in the process, the community benefits from analysis of numerous data sets which will be shared online in a poster format produced by the students at the end of their project. We present here the student posters from a pilot study on addiction, creativity, and memory.

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21. Integrating convergent evidence across species to identify conserved genes underlying behavior.

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Behavioral traits are phenotypically rich and genetically complex. Identifying the genomic features responsible for this phenotypic diversity can lead to a greater understanding of the mechanisms of behavioral variation in normal and pathological states. The plummeting cost of genome wide studies and the diversity of organisms with sequenced genomes has resulted in a proliferation of behavioral genetic and functional genomic data that can be used to understand behavioral variation within and across species. A major challenge for researchers lies in assembling and harmonizing this heterogeneous data across species. GeneWeaver is a user oriented database and analysis system for integrative

functional genomics that allows for the analysis of heterogeneous data across ten species. The set-set matching tools are based upon graph algorithms, and process large data sets, in real time, on the web. One way this integrated data can be utilized is to narrow QTL intervals through aggregate functional and genetic information. Behavioral traits such as activity, have been measured in a variety of species. By bringing together the QTL for comparable traits from multiple populations within and among diverse species, a QTL interval can be dramatically reduced. At the same time the features remaining within the interval can be prioritized by being 'annotated' with functional evidence such as; gene mutation studies, co-expression networks, ontological associations, GWAS, proteomic and metabolomics studies. Through this and other applications of the GeneWeaver system, heterogeneous functional genomic data can be applied to understand the biological basis of behavioral variation.

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Support: NIH R01 AA18776

23. Targeting Neuroimmune Pathways Reduces Alcohol Withdrawal Symptoms

Jessica A. Groot, Patrick C. Marquardt, Joseph M. Martinez, Clay L. Allison, David C. Curtis, Samuel J. Groot, and Susan E. Bergeson.

Withdrawal signs, including seizures, associated with Alcohol Use Disorder (AUD) can lead to a medical emergency. Every year withdrawal from ethanol consumption cessation affects hundreds of thousands of patients with AUD. Currently, the standard of care is treatment with benzodiazapines, which have co-addiction complications. We recently showed that neuroinflammation plays a role in alcohol consumption. A bioinformatics analysis of brain transcriptome data from control and alcohol treated mice found a significant neuroimmune component was associated with withdrawal seizures. To build on the exciting new finding that tetracycline analogs block neuroinflammation, we used tigecycline to test the hypothesis that the drug would reduce alcohol seizures. An injection of 4 g/kg i.p. ethanol together with vehicle or tigecycline (0, 20, 40 or 80 mg/kg i.p.) was administered to adult male and female DBA/2J mice simultaneously. Handling-induced convulsions (HIC) were determined before injection and then hourly for 12 consecutive hours. HIC score areas under the curve (AUC) were calculated for all mice. To test the effect of tigecycline on pharmacokinetic elimination of ethanol, blood ethanol concentrations (BEC) were determined at 2, 4, and 7 hours after ethanol injection for control 80 mg/kg treated mice. When compared to controls, animals given tigecycline showed a decrease in alcohol withdrawal symptom duration, peak magnitude, and overall severity. The dose response curve showed linear efficacy and BECs were not altered by tigecycline during its elimination. Our results suggest that tigecycline has exciting potential as a new pharmacotherapy for the treatment of alcohol withdrawal.

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Support: The Laura W. Bush Institute for Women's Health, and the Bryan C. Miller, Jr. and Martha H. Miller Foundation.

25. The International Mouse Phenotyping Consortium – a comprehensive functional catalogue of a mammalian genome catalogue

ME Stewart[1], SE Wells[1], T.Meehan[2], A-M Mallon[1,] MPI2 Consortium

The International Mouse Phenotyping Consortium (IMPC) is building the first truly comprehensive functional catalogue of a mammalian genome by producing and characterizing a knockout mouse strain for every protein-coding gene. Data from a standardized, broad-based phenotyping pipeline is collected and archived centrally by the IMPC-Data Coordinating Center. Dedicated 'data wranglers' are working with each phenotyping center to ensure proper transfer and quality control of data. A sophisticated statistical analysis pipeline identifies knockout strains with significant changes while accounting for bias from confounding effects. Annotation with biomedical ontologies allows biologists and clinicians to easily find mouse strains with phenotypic traits relevant to their research and facilitates integration with other resources. The IMPC resource adheres to the Animal Research: Reporting of in Vivo Experiments (ARRIVE) guidelines, which lay out the reporting requirements to ensure all the information is available to allow reproducible research and minimise overlap in strain production. MRC Harwell has been a key player in the IMPC project and is aiming to complete generating and phenotyping 520 lines in the first phase. With phenotype data now available from all the centres for over 3000 genes at mousephenotype.org, we will focus on the new insights the IMPC is providing in neuroscience not only in identifying novel gene function but also identifying new functions for known genes.

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Support: U54 HG006370, Medical research Council UK and NIH U42OD011174 and U54HG006348

27. Combined consumption of alcohol and a high-fat diet: effects on behavior and health

RR Gelineau, NL Arruda, JA Hicks, I De Pina Monteiro, A Hatzidis, JA Seggio

Poor diets and alcohol are known to affect both behavior and overall health. Each by itself can produce increases in anxiety and can affect insulin and leptin, important indicators of health. This project investigated how combined high-fat and alcohol access affected feeding and drinking behaviors, anxiety-like behaviors, and leptin and insulin levels in C57BL/6J mice. Mice were separated

into three groups: 60%-fat diet, 10%-fat diet, and regular chow and each group was paired with either water or forced 10%-alcohol. Weekly body weight, and food and drink intake measurements were recorded. Anxiety-like behaviors were measured using the open-field and light-dark box. Hedonic substitution was tested to determine if the addition of a 60%-fat diet or alcohol would affect alcohol or diet preference, respectively. Mice consuming 60%-fat diets exhibited increased insulin and leptin levels, but alcohol consumption had no effect on these hormones. In the open-field, there were differences between the 60%-fat and regular chow with respect to explorative behaviors, but not between the 60%-fat or 10%-fat diets; there were no differences among the groups in the light-dark box test. Alcohol had no effect on open-field or light-dark box behaviors. During some weeks the combined 60%-fat diet/alcohol mice drank more fluid than their water-consuming counterparts, a result not seen in regular chow and 10%-fat diet consuming mice, but there was no evidence of hedonic substitution during the preference tests. These results suggest that consumption of high-fat diets are more powerful in affecting behavior and health compared to moderate alcohol consumption.

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29. Assessing motivational drive to attain alcohol in *Drosophila melanogaster*

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Understanding alcohol's complex effects on reward and motivation circuits in the brain is critical for the development of better biologically informed therapies for alcohol abuse and addiction. With powerful neurogenetic tools, *Drosophila melanogaster* has emerged as an exciting model to study the effects of alcohol at the circuit and single neuron level. In order to determine how alcohol affects neural circuits and behavior, we have developed new assays for investigating motivational drive for alcohol. Preliminary results show that *Drosophila* can demonstrate both seeking and avoidance behaviors for alcohol or alcohol associated odors. Future studies will assess the necessity and sufficiency of specific neuronal circuits in alcohol mediated seeking and avoidance. These experimental paradigms for estimating motivational drive will allow for circuit and single neuron analyses of alcohol's effects. Our results will help inform highly similar and conserved circuit motifs in mammalian models.

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31. Genome-wide mapping of ethanol sensitivity in the Diversity Outbred mouse population

Steven Kasparek[1], Troy Wilcox[2], Daniel M. Gatti[2], Eric Busch[3], Emily Funsten[1], Drew Kreuzman[1], Benjamin Mansky[1], Sophie Masneuf[3], Erica Sagalyn[3], Kayvon

Sharif[1], Dominik Taterra[1], Walter Taylor[1], Mary Thomas[1], Andrew Holmes[3], Elissa J. Chesler[2], Clarissa C Parker[1]

A strong predictor for the development of alcohol use disorders (AUDs) is altered sensitivity to the intoxicating effects of alcohol. Individual differences in initial sensitivity to alcohol are controlled in part by genetic factors, yet finding specific genes underlying these differences has proven difficult. Mice offer a powerful tool for elucidating the genetic basis of behavioral and physiological traits relevant to AUDs; yet conventional experimental crosses have only been able to identify large chromosomal regions rather than individual genes. Genetically diverse, highly recombinant mouse populations allow for the opportunity to observe a wider range of phenotypic variation, offer greater mapping precision, and thus increase the potential for efficient gene identification. We used the newly developed Diversity Outbred (DO) population (n ~ 783) to fine-map quantitative trait loci (QTLs) associated with three measures of ethanol sensitivity: ethanol-induced ataxia, ethanol-induced hypothermia, and ethanol-induced loss of the righting response (LORR). We used the GIGAMUGA to genotype a subset (N = 288) of these mice at ~140k SNP markers across the genome and performed high precision QTL mapping using DOQTL. Our preliminary QTL analyses identified numerous suggestive QTLs associated with ethanol sensitivity on chromosomes 6, 7, 8, 9, 12, 15, 16 (LODs > 5; $p < 0.05$) and one significant QTL located on chromosome 11 (LOD > 8; $p < 0.05$). This information can in turn be used to identify alleles that contribute to AUDs in humans, elucidate causative biological mechanisms, or assist in the development of precise preclinical models and putative treatment strategies.

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33. Functional *in vitro* studies within CHRNA5-CHRNA3-CHRNB4 gene cluster on Chr15q24-25.1 locus

Belimezova S, Galego XG, Myamoto-Ditmon J, Stitzel JA, & Ehringer MA (2016)

Polymorphisms on human chromosome 15 located in *CHRNA5, CHRNA3* and *CHRNB4* genes have been widely associated with smoking (nicotine addiction), lung cancer, COPD, cocaine addiction, and alcohol dependence. These genes encode for the alpha5, alpha3 and beta4 subunits of nicotinic acetylcholine receptors (nAChRs), respectively, whose gene expression is tightly regulated. Our study focuses on non-coding variants (SNPs) and potential transcription factor binding sites and/or noncoding eRNA transcripts within *CHRNA5* distal upstream (proposed enhancer/repressor) and core promoter regions that have been shown to regulate *CHRNA5* mRNA expression: rs3841324, rs880395,

rs905740, rs7164030. The project involved cloning the enhancer/repressor (distal regulatory) and promoter regions of *CHRNA5* (total of 7 different plasmid constructs) and quantifying differences in gene expression using a Luciferase assay. The latter has been completed in two human neuroblastoma cell lines [BE(2)-C and SHSY-5Y, ATTC] and in one human-derived small cell lung carcinoma cell line [H446, ATTC]. Our results so far suggest that the 22bp-indel polymorphism (rs3841324) in the core *CHRNA5* promoter region is a main modulator of the luciferase expression activity in the context of our experimental design. An electromobility shift assay analysis of the 22bp-indel *CHRNA5* promoter segment showed a positive shift in all three studied cell lines, and was performed as a first step of a more extensive proteomic study of the aforementioned promoter and upstream distal regulatory regions of interest. Our next step will be to identify the specific functionally important DNA-binding proteins (TFs) and their corresponding TF-binding sites. This work has the potential to identify novel targets for therapeutics aimed at reducing smoking behaviors.

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Support: NIDA - T32 DA017639, NIH - R01 AA071889

35. Use of the Visual Cliff apparatus for high-throughput quantification of impulsivity

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Behavioral traits including impulsivity, risk-taking, response to novelty, and anxiety are associated with addiction related behaviors in humans and experimental animals. We and others have shown that these behavioral associations are driven by shared genetic mechanisms. We have begun to leverage the genetic overlap among addiction and associated behavioral traits to identify genes driving addiction using a high throughput approach. For years, this approach has been amenable to identification of genes underlying novelty and anxiety traits because indexing these traits is relatively simple, rapid, and cost effective in mice. In contrast, characterization of impulsivity is a relatively time consuming and expensive process. Currently, the most commonly used impulsivity screen is the 5-choice serial reaction time task which requires a 3 – 4 month testing protocol using an operant conditioning chamber. In order to facilitate the prospective identification of genes underlying impulsivity, it is necessary to create a high throughput, non-invasive, and inexpensive impulsivity assay. To that end, we have evaluated the use of the Visual Cliff apparatus to index impulsivity in the laboratory mouse. We tested male and female mice from 64 BXD strains on the Visual Cliff apparatus. We calculated heritability of Visual Cliff phenotypes, identified quantitative trait loci associated with Visual Cliff phenotypes, and identified genetic correlations among Visual Cliff traits,

neurochemical correlates, and addiction-relevant behaviors. Collectively, these findings show covariation among behaviors in the Visual Cliff and behaviors and genetic mechanisms associated with drug use.

37. Sex differences in cognitive and behavioural tasks in the 5xFAD mouse model of Alzheimer's Disease

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Alzheimer's Disease (AD) is the leading cause of dementia, and is brought on by an abnormal accumulation of amyloid-β peptides in the cerebral cortex. In order to provide a better understanding of the epidemiology of the condition, several mouse models have been developed, including a transgenic mouse model with five familial AD mutations (5xFAD). These mutations result in a rapid accumulation of 42-amino acid amyloid-β peptides in the cerebral cortex, hippocampus, and spinal cord, with neurodegeneration evident by four months of age. By 6 months of age, transgenic mice exhibit a number of behavioural and cognitive deficits. This study examines the sex differences in 5xFAD mice in a number of cognitive and behavioural tasks (including sensory perception, motor behaviour, cognitive ability, and social behaviour), as well as examining the impact of genotype (wildtype vs transgenic) on the sex differences. Results indicate that female mice in general perform better than males on olfactory matching-to-sample tests, grid- and wire-suspension tasks, and had more spontaneous alternations and arm entries in a Y-maze; males performed better on the Morris Water Maze (cumulative search error, percentage of time in correct quadrant, average proximity to escape platform, and latency to find escape platform), and improved more over successive days of training with the visual water box. Female transgenic mice exhibited more centre entries and a larger distance travelled in the open-field maze, and have lower scores on the frailty index than male transgenic mice. These results demonstrate the importance of testing transgenic mice of both sexes in behavioural studies.

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39. Removal of a high-fat diet produces in improvements anxiety-like behavior and health

I De Pina Monteiro, NL Arruda, JA Hicks, RR Gelineau , A Hatzidis, JA Seggio

Consumption of high-fat diets can lead to deficits in cognitive behaviors and increases in anxiety, in addition to producing obesity and other health problems, as seen in both human and animal studies. This study investigated the effects of high-fat diet removal on anxiety and obesity measures in C57BL/6J mice. Initially, mice were given access to either regular chow or 60%-fat diets and after five weeks on their respective diets, their anxiety-like behaviors were observed using the open-field and light-dark box tasks and their insulin and leptin levels were measured. After the baseline measurements, half of the mice given 60%-fat diets

had their diets replaced with regular chow, and after another five weeks, their anxiety-like behaviors and hormone levels were measured again. Prior to the switching of the high-fat diet to regular chow, all high-fat diet mice exhibited similar levels of insulin and leptin to each other and significantly increased levels compared to mice consuming regular chow. Additionally, high-fat diet consumption also produced changes in behavior during the open-field, but not the light-dark box. Removal of the high-fat diet significantly improved body mass and hormone levels compared to mice on continuous high-fat diet access and reduced anxiety. In summary, the mice with high-fat diet replaced with regular chow exhibited similar anxiety-like behaviors and health compared to regular chow controls. These results indicate that replacement of a poor diet with a healthier one can produce improvements in anxiety and overall health.

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41. Genetic Mapping in Diversity Outbred mice identifies a novel *Trpa1* functional variant affecting inflammatory pain sensitivity

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Chronic pain response is a complex behavioral trait with known genetic variability in humans and mice. Identifying genetic variants underlying complex behavioral traits in mice has been historically limited by low mapping resolution of conventional mouse crosses. The Diversity Outbred (DO) population offers increased genetic heterozygosity and allelic diversity compared to crosses involving inbred mouse strains. DO mice are derived from the same eight inbred founder strains as the Collaborative Cross, including three wild-derived strains. We characterized inflammatory pain sensitivity in 275 male and female DO mice using the formalin assay of tonic chemical nociception. One quantitative trait locus (QTL) reached genome-wide significance with a support interval of 2.23 cM. This locus, which we have named *Nociq4* (nociceptive sensitivity inflammatory QTL 4; MGI:5661503), harbors the well-known pain-related gene *Trpa1* (transient receptor potential cation channel, subfamily A, member 1). Using the founder strain haplotype-predicted allelic effects together with whole genome sequence data, we identified a *Trpa1* functional variant private to the CAST/EiJ mouse strain (Val115Ile). Electrophysiological data from Val115Ile-transfected HEK cells were used to assess functional effects of the *Trpa1* CAST/EiJ allele. Expression effects of the Val115Ile allele were examined by comparing transcript abundance of *Trpa1* in the dorsal root ganglia of CAST/EiJ and C57BL/6J mice. Our results demonstrate that high-precision mapping of pain-related traits can be achieved with moderate numbers of DO animals, representing a significant advance in our ability to leverage the mouse as a tool for the discovery of pain-related genes and therapeutic targets.

[1] The Jackson Laboratory, Bar Harbor, ME, USA, [2] IGERT Program in Functional Genomics, Graduate School of Biomedical Sciences and Engineering, The University of Maine, Orono, ME, USA **Support:** This work was funded in part by DOD grant W81XWH-11-1-0762 (CJB), USA.

43. Neurobiological mechanisms of hnRNP H1 in methamphetamine addictive behaviors

Neema Yazdani[1,2], Eric R. Reed[5], Qiu T. Ruan[2], Michael Chau[1], Farzad Mortazavi[3], Douglas Rosene[3], W. Evan Johnson[4], Camron D. Bryant[1]

Using fine mapping and gene editing, we recently identified *Hnrnph1* (heterogeneous nuclear ribonucleoprotein H1) as a quantitative trait gene for the locomotor stimulant properties of methamphetamine (**MA**) (*PLOS Genetics*, 1(12):e1005713). To extend the contribution of *Hnrnph1* to MA reward, we conducted conditioned place preference (**CPP**) at multiple doses, which consisted of one day of baseline preference assessment, four alternating saline and MA training days on blunt or sharp floor contexts, two consolidation days, and assessment of MA drug preference on Day 8. According to Day 8 vs. Day 1, *Hnrnph1*$^{+/-}$ mice were significantly less sensitive to the rewarding properties of MA at 0.5 mg/kg (i.p.). Transcriptome analysis of *Hnrnph1*$^{+/-}$ striatal tissue (100 bp paired-end sequencing) followed by Ingenuity Pathway Analysis (**IPA**) revealed perturbations in EIF2 signaling (global protein translation), PKA signaling, and axon guidance signaling in neurons, among others. Spliceome analysis followed by IPA revealed perturbations in GABA receptor, glutamate receptor and alpha-adrenergic signaling. Diseases and biofunctions analysis for both datasets ranked neurological disorders as a top category which clustered genes into functional annotation groups including “movement disorders”, “disorder of basal ganglia”, and “degeneration of neurons.” Currently, we are conducting a thorough immunohistochemical (**IHC**) assessment of hnRNP H throughout adult WT B6 and *Hnrnph1*$^{+/-}$ brains. Preliminary results illustrate pan-neuronal expression of hnRNP H and exclusion in glia. There was also a trend for an increase in striatal glia in *Hnrnph1*$^{+/-}$ brains. Thus far, we hypothesize hnRNP H1 to be vital in the development and function of midbrain dopaminergic neurons and glia, which is necessary for the rewarding and stimulant properties of psychostimulants.

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Support: K99/R00DA029635 (C.D.B.), R01DA039168 (C.D.B.), R01HG005692 (W.E.J.), T32GM008541 (D.H.F.), TTPAS Burroughs Wellcome Fund (N.Y.)

45. Predictive identification of addiction related genes using rapid behavioral screening of drug-naïve knock-out mice

T. D. WILCOX[1], T. A. ROY[1], P. E. DICKSON[1] J. NDUKUM[1], J. W. CLARK[1], J. A. BUBIER[1], S. J. SUKOFF RIZZO[1], J. C. CRABBE[2], J. M. DENEGRE[1], K. L. SVENSON[1], R. E. BRAUN[1], V. KUMAR[1], E. J. CHESLER[1];

There is substantial evidence that predisposing behaviors predict subsequent addiction and there is high prevalence of dual diagnosis of addiction, alcoholism and other disorders. However, such relations are difficult to assess in high-throughput settings because drug exposure, physiological and behavioral testing interfere with one another. Therefore we sought to identify relevant addiction genes using data from drug-naïve mice from the KOMP 2 behavioral phenotyping pipeline. Nineteen strains that exhibited significantly different phenotypes relative to C57BL/6NJ controls on at least one behavioral assay with relevance to anxiety, depression, or response to novelty were chosen to test the predictive validity of these assays for alcohol and drug preference and consumption related phenotypes. These strains were tested on three self-administration assays: nicotine two bottle choice, methamphetamine two bottle choice, or ethanol two bottle choice. We predicted that anxiety, reactivity and depression related behaviors will predict increased alcohol consumption, anxiety and depression behaviors will predict increased nicotine consumption, and novelty response/exploratory behavior mutants will predict increased methamphetamine consumption. Results indicate that knock-out strains with significant predisposing behaviors often, but not always had significantly different drug self-administration relative to C57BL/6NJ controls. This study demonstrates the promise of predictive validity of drug-naïve behavioral assays in the JAX KOMP 2 phenotyping pipeline for the discovery of novel alcohol- and substance use-related genes.

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Support: NIH Grant# U54 HG006332

47. Quaking -Moving from human to fish to understand the connection between glia cells, myelination, synaptogenesis and higher brain function

Lina Emilsson

Quaking (*QKI*) is a gene exclusively expressed in glial cells, with downregulation in schizophrenic patients (Radomska et al. HMG 2013, Ng et al., Mol Psychiatry 2009, Åberg et al., PNAS 2006; Lindholm et al., AJHG 2001;) and an upregulation in Alzheimer's disease patients (manuscript submitted to Journal of Alzheimer's disease). The embryonic lethality of *Qk* knockouts in mice and the characteristic "quaking" of the hindquarters in the viable mutant lines have hampered detailed functional analysis of this gene. However, early expression

changes of QKI have shown developmental changes in *Qk* mouse mutants. To target the functions of *QKI*/*Qk* homologs during early development we have turned to the zebrafish. We have shown that *qkia* is a paralog, while *qki2* and *qkib* are orthologs of the human *QKI* gene (Radomska et. al. Plos One 2016). Furthermore, we have shown that both *qki2* and *qkib,* but not *qkia*, were expressed in the progenitor domains of the central nervous system, with overlapping yet unique expression patterns, indicating subfunctionalization following gene duplication. In preliminary morpholino experiments, *qkib* knockdown has shown alterations to oligodendrocyte precursor cells and morphological changes to olig2 positive developing motor neurons and eurydendriod cells in the cerebellum, while *qki2* showed effects on mature myelin-forming glia. As a next step we are addressing these findings by using CRISPR /Cas9 transgenic fish, molecular, cellular and behavioural experimental setups. Our end goal is to understand how developmental aspects of *qki* affects learning outcomes.

Uppsala University, Uppsala, Sweden

49. Sensitivity of a novel paradigm for detecting episodic-like memory impairments in 5XFAD and aged mice.

SJ Sukoff Rizzo[1] and L Leventhal[2]

Episodic memory is the memory of a specific past event that is recalled in the context of a particular place at a particular time, and in association with contextual information such as emotional responses, semantic knowledge, and olfactory, auditory, and/or visual cues (Dere et al 2006; Eacott and Easton 2010; Eichenbaum et al. 2012; Kim et al 2015). While episodic memory function has been reported to decline with healthy aging similar to other cognitive processes, it has also been reported that some of the earliest impairments observed in Alzheimer's (AD) patients are episodic memory deficits. In the present studies, a novel episodic-like memory paradigm was developed using a Y maze that requires subjects to discriminate incongruency of a visual cue, in a specific location/arm of the maze, in relation to a specific visual-tactile context. Evaluation of C57BL/6J mice revealed an age-related decline whereas episodic-like memory was intact in naïve 3 and 6 month aged mice, but impaired in 12 month aged mice. Acute administration of scopolamine also impaired episodic-like memory in this task, relative to vehicle treated controls. Longitudinal evaluation of 6 month aged 5XFAD (B6.Cg-Tg(APPSwFlLon,PSEN1*M146L*L286V)6799Vas/Mmjax #008730) mice revealed impairments in episodic-like memory in HEMI mice at 6 months of age while intact memory was observed in age-matched WT controls. At 9 months, both WT and 5XFAD mice demonstrated impairments in episodic-like memory, consistent with the expected age-related decline. Taken together the present data demonstrate the validity of a novel Y maze paradigm for evaluating episodic-like memory deficits in mice.

[1]The Jackson Laboratory Mouse Neurobehavioral Phenotyping Facility Bar Harbor Maine USA

51. The JAX repository of mouse models for neurobehavioral genetics
M Sasner, A Valenzuela, J Beckwith, SF Rockwood

The JAX mouse repository provides over 8,000 distinct genetic models, many of which can be used to study the role of genetic factors on specific behaviors. Many genetically engineered models display alterations in various behaviors. Recombinant inbred strain panels can be used to correlate genetic factors to behavioral phenotypes such as alcohol preference. The Diversity Outbred and Collaborative Cross panels are tools for high resolution mapping of QTLs. Dozens of strains expressing optogenetics effector proteins that can be used to study neural mechanisms of behavior are also available. Opsins are light-activated proteins that alter membrane potential in neurons, so that light stimulation allows control of neuronal activity. Several mouse models express improved/optimized opsins fused to fluorescent proteins, including channelrhodopsin, archaerhodopsin, halorhodopsin variants with their expression directed by specific promoters.
Many of these are expressed in a *cre*-dependent manner, enabling a novel model to be created by simply mating a strain expressing *cre* in a specific neural cell type to a strain carrying a floxed-stop effector molecule. Neural activity can be controlled or monitored using an activating channel such as channelrhodopsin, an inhibitory channel such as halorhodopsin, or a calcium- or voltage- indicator. Repository holdings are easily searched using the newly designed JAXMice webpage (**jaxmice.jax.org/query**). To donate mouse strains, use our online submission form (**jax.org/donate-a-mouse**).

Genetic Resource Science, The Jackson Lab, Bar Harbor, ME, USA
Support: The JAX Repository is supported by NIH, The Howard Hughes Medical Institute and private foundations.

53. Central amygdala nociceptin neurons inhibit high fat food consumption
JA Hardaway[1,4], M Kim[1,4], CM Mazzone[1,4], JR Jensen[1,4], LB Brogden[1,4], A Shiddapur[1,4], JA Sugam[1,4], CM Bulik[2,3,5] & TL Kash[1,4]

Feeding is a complex phylogenetically-conserved behavioral program that is regulated by a balance of reward and metabolic stop-and-go signals encoded within discrete neural circuits located in brain regions like the hypothalamus, hindbrain, brain stem, and amygdala. Within the central amygdala (CeA), neurons coexpressing opioid-like neuropeptides and fast acting neurotransmitters like GABA are activated during feeding, but the functional role of these genetically-defined neurons is still unclear. We identified a novel population of neurons in the CeA that express the orexigenic heptadecapeptide nociceptin. Using Prepronociceptin–*IRES*-Cre mice paired with immunohistochemical (IHC) detection of the immediate early gene *c-fos*, we

found that CeA^{NOC} neurons are activated following intermittent 1 hour access to palatable high fat food that promotes binge-like eating. To dissect the functional role of these neurons in binge eating, we used a suite of viral-mediated Credependent tools encompassing a proteolytic Caspase complex and excitatory/inhibitory DREADDs. Together, our data demonstrate that CeA^{NOC} neurons inhibit intermittent access binge eating of high fat food. Surprisingly, however, we found that persistent ablation of CeA^{NOC} neurons renders these animals resistant to diet-induced obesity under continuous access to high fat food. CeA^{NOC} neurons send axons to the BNST, PBN, and NTS. Injection of the retrograde tracer cholera toxin in these postsynaptic sites combined with *c-fos* IHC in the CeA revealed that CeA^{NOC} neurons that project to the NTS and PBN are activated following high fat binge eating. We are currently pursuing functional assessment of these pathway specific outputs using optogenetic and intersectional DREADD approaches.

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Support: J.A.H was funded by MH076694. T.L.K. acknowledges funding by AA011605, AA019454, AA020911, MH105892. C.M.B acknowledges funding from the Swedish Research Council (VR Dnr: 538-2013-8864) and a grant recipient from Shire pharmaceuticals.

55. Genetic Influences on Resting EEG Alpha Power in an American Indian Tribe

M-A Enoch[1], CA Hodgkinson[1], P-H Shen[1], Q Yuan[1], D Goldman[1]

The EEG records the rhythmical, electrical activity of the brain that varies with mental activity, relaxation, drowsiness and sleep. As an indicator of cortical activation, the highly heritable resting EEG may be regarded as an intermediate phenotype for arousal-related behaviors such as anxiety and addiction. Therefore candidate genes are likely to be stress-related. This study focused on alpha power (8-13Hz) that is maximal during eyes-closed relaxation and mental inactivity.
Participants included 308 Plains American Indians: mean(SD) age = 42.8(12.9), range = 18 - 87 years, 58% women. Eyes-closed, normalized resting EEG spectral power was obtained. Twenty common, putatively functional polymorphisms in stress-related genes implicated in the HPA axis, GABAergic, serotonergic, dopaminergic and cannabinoid systems were selected. Genotypes were available from an array using the Illumina GoldenGate platform and from a GWAS, using the Illumina HapMap550K.
Alpha power decreased with age ($p < 0.005$). A regression analysis revealed that there were independent effects of *CRHBP*, *GPHN*, *SLC6A4*, *HTR3B* and *FAAH* functional polymorphisms on alpha power that together accounted for 13% of the variance. The *FAAH* SNP rs324420 had the strongest effect. In all cases, the

minor allele/homozygote was associated with higher alpha power. Moreover, there was a gene dosage effect: the number of minor allele/homozygotes per individual was positively correlated with alpha power.
Our study has shown that variation in stress-related genes predicts alpha power. Since the minor allele/homozygotes were associated, much larger samples are required to investigate the trend significant results for some polymorphisms including in *NPY*, *CRH* and *GABBR1*.

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57. Identifying novel behavioural mouse models from the International Mouse Phenotyping Consortium Resource.
J Williams, MM Simon, G Banks, S Greenaway, S Sethi, H Morgan, L Santos, T Fiegel, N Ring, H Westerberg, G Yaikhom, PM Nolan, S Wells, SDM Brown and A-M Mallon

The International Mouse Phenotyping Consortium (IMPC, http://www.mousephenotype.org) is a large scale effort to produce and characterize a knockout strain for every protein coding gene, building the first truly comprehensive functional catalog of a mammalian genome. IMPC has established a high-throughput broad based standardized phenotyping pipeline (http://www.mousephenotype.org/impress) that will assess different aspects of mouse biology. Data from this pipeline are collected and archived for both male and female mice by the IMPC-Data Coordinating Center. Dedicated 'data wranglers' coordinate with each phenotyping center to ensure proper transfer and quality control of data. A sophisticated statistical analysis pipeline identifies knockout strains with significant changes while accounting for bias from confounding effects. To date the project has generated data for nearly 4000 genes.
The pipeline includes a number of robust neurobehavioral phenotyping procedures that to date have produced a number of phenodeviants from the statistical pipeline. This collection of both known and novel mutants with phenodeviancy in one or more test, were further analyzed with expert scientists to ascertain a robust collection of mutants. This collection was further analyzed with additional datasets such as transcriptomics providing novel insights on gene function.

Medical Research Council Harwell (Mammalian Genetics Unit and Mary Lyon Centre), Harwell, Oxfordshire, OX11 0RD, UK.

59. Substrain differences in ethanol preference and running wheel activity in C57BL/6J and C57BL/6N mice.
WD McCulley III[1], M Hartmann[2], SE Holbrook[3], V Kumar[4], AM Rosenwasser[1, 2, 3]

The C57BL/6 (B6) mouse is the most widely used inbred strain in biomedical research, and has served as the basis for multiple large-scale genetic and

genomic projects. While the B6 mouse originated at The Jackson Laboratory, a number of separate breeding colonies are now maintained at various sites, and genetic drift has led to the emergence of both genotypic and phenotypic differences among these colonies. Researchers currently recognize two distinct substrains of B6 mice, the C57BL/6J (B6J) and the C57BL/6N (B6N) ("J" for Jackson Laboratory and "N" for the National Institute for Health, respectively). B6 mice are well-known to display high levels of alcohol and drug self-administration, making them popular subjects for substance abuse research. Nevertheless, B6J mice are reported to display higher levels of ethanol preference and increased locomotor responses to psychostimulants relative to B6N. In the present study, we assessed ethanol preference drinking (2-bottle choice) and running-wheel activity in male and female B6J and B6N mice from The Jackson Laboratory. Mice were maintained individually in running wheel cages under light-dark 12:12 conditions for 2 weeks prior to the introduction of ethanol access, and then allowed access to increasing concentrations of ethanol solution from 3 to 21% for 5 days at each concentration. Male and female B6J mice displayed higher levels of running-wheel activity and ethanol preference relative to B6N mice. Subsequent research will examine whether these phenotypic differences depend on similar or different genetic polymorphisms relative to those suggested to underlie the substrain difference in cocaine response.

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61. Illustrating psychiatric disease classification and overlap through GeneWeaver geneset associations

Timothy Reynolds[1], Jason Bubier[2], Elissa J Chesler[2*], Erich J Baker[1,4*], and Michael Langston[3]

Disease classification among psychiatric disorders is often problematic due to a substantial overlap among observed conditions and complex interactions among genetic components. In order to mitigate potential bias rooted in shared symptomatology, there is an increasing movement to base classification strategies on the underlying biology. Using GeneWeaver we collected bipartite intersections between sets of genes manually curated to a wide range of behavioral and psychiatric disorders, spanning nine model organisms. In addition, sets of genes were curated to psychiatric-related MeSH terms by examining consensus gene and MeSH relationships in the Pubmed literature. Here, we investigate communities of bipartite graphs for conserved gene to geneset associations. Identifying the extent to which sets of genes are associated to controlled vocabularies can be a powerful means to test our assumptions about the conservation of nosology. This includes the potential ability to quantitate the veracity of semantic term annotations and discovery of novel and overlapping behavioral disease categories. We illustrate the ability of

the GeneWeaver data and analysis environment to identify common and unique disease classifications based on functional genomics data and genes shared in curated literature.

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63. EAAT2 Regulation as a Mechanism for NRG3-Mediated Nicotine Withdrawal Phenotypes

Adewale Adeluyi and Jill Turner

A substantial percentage of adult smokers attempting to quit fail and this poor quit rate is thought to be due to nicotine's effects on plasticity mechanisms in the brain. These effects, which occur during the process of drug addiction, are transcriptionally dependent and lead to various forms of cellular adaptation, including alterations in mRNA and protein expression patterns. Excitatory amino acid transporters (EAATs) are membrane-bound transport proteins with a well-defined role in the removal of extracellular glutamate from the synaptic cleft. As altered clearance of extracellular glutamate has been shown to be a hallmark of addiction, regulation of these proteins is both a potential mechanism of relapse susceptibility as well as a potential therapeutic target. While little is known regarding EAAT regulation, recent research has suggested that there is an interaction between certain EAATs and Neuregulin signaling. Published studies from our lab have demonstrated a genetic link between Neuregulin 3 (NRG3) and smoking cessation success; therefore, examination of NRG3 regulation of EAATs during nicotine dependence and withdrawal may be of immediate interest in nicotine addiction. From our recent studies, we found that EAAT2 mRNA, whose protein is responsible for ~80% of glutamate clearance, is significantly upregulated following nicotine withdrawal in the striatum of mice with reduced Nrg3 levels. Interestingly, these mice do not present with many nicotine withdrawal symptoms, suggesting that regulation of EAAT2 may be a causal link. Therefore, our study further examines the interaction of EAAT2 with NRG3 in the development of NRG3-mediated nicotine dependence.

Department of Drug Discovery and Biomedical Sciences, South Carolina College of Pharmacy, University of South Carolina, Columbia, South Carolina

Support: NIDA 1-R00-DA032681

Saturday May 14, 2016

Young Scientist: Alex Keene

Genetic and evolutionary dissection of the sleep-feeding conflict.

Keene, Alex C.

Animals modulate sleep in accordance with metabolic state, yet the molecular basis for this interaction remains poorly understood. Our research program employs genetic approaches to investigate the integration of sleep and metabolic state. Starved flies suppress sleep, presumably to increase foraging opportunity. We have performed a large-scale RNAi screen and identified the RNA-binding protein TRANSLIN (TRSN) as essential for suppressing sleep during starvation. Spatially restricted rescue or targeted knock down localizes *trsn* function to neurons that produce the tachykinin-family neuropeptide Leucokinin. These findings suggest *translin* and Leucokinin are critical integrators of sleep and metabolic state. Additionally, we are investigating the evolution of sleep in the Mexican cavefish, *Astyanax mexicanus*, that evolved in nutrient-poor environments. *A. mexicanus* exist as isolated populations consisting of an ancestral eyed surface morph and numerous blind cave morphs of the same species. The extreme differences in habitat between surface and cave populations of *A. mexicanus* present a unique opportunity to examine the consequences of ecology and evolutionary history on sleep. We have previously demonstrated the convergent evolution of sleep loss in multiple independent *A. mexicanus* cave populations. Our recent findings reveal that increases in the number of mechanoreceptive lateral line neuromasts in cavefish underlie sleep loss and promotes expression of *Orexin*, a highly conserved sleep-suppressing neuropeptide. These findings demonstrate that evolutionarily derived changes in sensory processing contribute to sleep regulation.

Florida Atlantic University, Biological Sciences, Boca Raton, FL

Symposium 2: Analysis of 3D genomes and chromatin

Annotation of non-coding regulatory elements via 3D chromosome conformation in human brain development

Hyejung Won[1], Luis de la Torre-Ubieta[1], Jason L. Stein[1], Neelroop N. Parikshak[1], Farhad Hormozdiari[3], Changhoon Lee[1], Eleazar Eskin[3,4], Jason Ernst[2,3], Daniel H. Geschwind[1,4]

Chromatin remodelers and architectural proteins regulate chromatin interactions to influence the landscape of gene expression. Chromatin contacts mediate gene regulation via physical interactions with non-coding regulatory elements and are critical for the differentiation of the developing telencephalon. Genetic variation associated with neuropsychiatric disorders, including autism and schizophrenia, is enriched within or nearby chromatin remodeling genes. In addition, most genetic variation associated with neuropsychiatric diseases is found within intergenic or intronic elements presumed to have a regulatory role despite that the gene of action is totally unknown.
Here we have obtained comprehensive landscape of chromosome conformation during early cortical development by performing Hi-C analysis on human fetal

cortical laminae. We first interrogated global folding principles of chromatin in neural tissue by integrating Hi-C data with transcriptomic and epigenomic data. We then utilized chromosome contact information to identify putative gene of actions for non-coding regulatory elements, including human-gained enhancers and common variants for schizophrenia.
Given that the 3D conformation of chromosomes functions as a blueprint for gene regulation, unveiling the folding principles of chromatin in early fetal brain development can provide a core rubric for annotation of non-coding regulatory elements as well as novel insights into the pathogenic mechanism of neurodevelopmental disorders.

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Genome in 3D: Exploring Spatial Genome Architectures and Function in Mouse and Human Brain

Amanda Mitchell and Schahram Akbarian

We will discuss how innovative neuroepigenetic approaches, including chromosome conformation capture and (epi)genomic editing informs about neurological function of non-coding DNA. We will provide specific examples, involving intergenic and intronic risk-associated polymorphisms of ion channel and neurotransmitter genes and draw a wider perspective how these types of approaches will complement current efforts by PsychENCODE and other consortia dedicated to map neuronal and non-neuronal epigenome across the lifespan of normal and diseased human and mouse brain. Furthermore, we will introduce novel chromosome conformation capture approaches, including ‘in situ Hi-C’, that will greatly complement related procedure such as ‘Hi-C’ because the former has much higher spatial resolution. Finally, we will discuss longitudinal approaches in the field, which are likely to provide an important complement to current approaches which are largely cross-sectional and provide only a snapshot of genome organization and function from the time of tissue harvest.
Acknowledgement:

Friedman Brain Institute, and Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York 10029

Support: NIDA R01 DA036984-03 and NIMH R01 MH093332-06 (S.A.) and the Brain & Behavior Research Foundation (A.M.)

3D chromatin architecture defines inherited disease risk

Olivia Corradin[1], Andrea J. Cohen[1], Jennifer M. Lupinno[1], Ian M. Bayles[1], Peter C. Scacheri[1,2]

DNA variants associated with susceptibility to common diseases through Genome Wide Association Studies (GWAS) often lie in transcriptional enhancer elements located distal to protein-coding genes. This enrichment is particularly pronounced in regions of enhancer clusters, where multiple active enhancers are arrange in close proximity to genes that regulate cell identity functions. Chromatin interaction studies have demonstrated that multiple constituents of enhancer clusters cooperate to regulate target gene expression. Given that multiple enhancer elements regulate a given gene, we reasoned that the phenotypic variance attributable to a given locus may be explained by interactions between variants that are found within these functional elements. We find that gene targets of GWAS associated SNPs are also frequently coupled with additional regulatory elements that contain novel 'outside variants' that are inherited independently of the GWAS association signal. Using an integrative approach to compare transcriptomic, and chromatin interaction datasets with patient genotypes, we demonstrate that outside variants cooperate with GWAS SNPs to modify the effect of the risk allele on target gene transcript levels and alter clinical risk to disease. Remarkably, outside variants increase disease heritability estimates by as much as an order of magnitude. Collectively these results demonstrate that genetic loci that contribute to disease heritability frequently involve an interplay of multiple functional variants that combine impact enhancer function, alter gene expression and define clinical risk to disease.

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3D genome architecture and topological framework of transcription regulation

Yijun Ruan

Three-dimensional (3D) genome organization and its effect on transcription remains a fundamental biological question. We developed and applied the ChIA-PET strategy[1] to comprehensively map higher-order chromosome folding and specific chromatin interactions mediated by CTCF[2] and RNAPII[3] with haplotype-specificity and base pair resolution in distinct human and mouse cells. We have found that CTCF-mediated chromatin interaction anchors serve as 3D organizational foci, where constitutive genes are positioned in concordance with the orientation of CTCF binding motifs, whereas RNAPII interacts within these structures by drawing cell-type-specific genes towards CTCF-foci for coordinated transcription[4]. In addition, we show that haplotype-resolved allelic-specific chromatin interactions have differential effects on chromosome configuration and

further influence the expression of genes residing in the topological domains. This may provide a topological mechanism, in which genetic variations occurred in non-coding distal elements could disrupt proper 3D chromatin organization and lead to altered molecular functions that are associated with disease susceptibility. Furthermore, 3D genome simulation suggests a model of chromatin folding around chromosomal axes, where CTCF define the interface between the condensed and open chromatin compartments for structural regulation of transcriptional activity. Together, these mechanistic insights provide an emerging topological paradigm to unify our understandings of non-coding distal regulation, enhancer function, complex traits and diseases.

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3. Li et al (2012) Extensive Promoter-centered Chromatin Interactions Provide a Topological Basis for Transcription Regulation. Cell 2012 Jan 20; 148: 84-98
4. Tang et al (2015) CTCF-Mediated Human 3D Genome Architecture Reveals Chromatin Topology for Transcription. Cell 2015 Dec 17; 163(7): 1611–1627

The Jackson Laboratory for Genomic Medicine 10 Discovery Drive, Farmington, Connecticut 06032

Symposium 3: Neurogenetics of Circadian Behavior, Sleep and Metabolism

Genetic Dissection of Neurons Coordinating Sleep-Wake Behavior and Metabolism

Annika Barber[1,2], Dan Cavanaugh[3], Amita Sehgal[1,2]

Circadian clocks play a key role in regulation of behavior and physiology, however the output pathways coupling the brain clock to outputs at the behavioral and molecular level are still poorly understood. The Sehgal lab has found that peptidergic cells of the *Drosophila pars intercerebralis* (PI), a functional homolog of the mammalian hypothalamus, are a component of the circadian output pathway. GFP reconstitution across synaptic partners (GRASP) shows that PI cells expressing the corticotropin-releasing factor (CRF) homolog, DH44 and *Drosophila* insulin-like peptides (DILP) are connected to the central pacemaker cells via a multisynaptic connection. Patch clamp electrophysiology has demonstrated that the *Drosophila* insulin producing cells (IPCs) show rhythmic diurnal firing with high firing frequency in the morning and lower frequency firing in the evening and late night. Importantly, DILP cells do not contain a clock themselves, and thus provide one of the first examples of firing rhythms driven by upstream clock neurons. Classically insulin release is closely tied to feeding, however here we found that the timing of feeding has limited effect on the DILP2 neuronal firing pattern, suggesting that activity of DILP2 cells of the PI is primarily regulated by the circadian clock. While DILP cells are likely

important for rhythms in metabolic function, DH44 is required for normal rest:activity rhythms in *Drosophila.* We propose that the PI integrates time-of-day information from clock neurons with environmental information from other sources, such as metabolic information sensed by IPCs, to coordinate behavior in response to multiple environmental cues.

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Funding Support: Howard Hughes Medical Institute, NINDS R37 NS048471, NINDS F32 NS090811

Mis-timed Feeding Causes Dysregulated Metabolism in Hypothalamic Circadian Mutant Mice

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Mis-timed feeding in relation to the sleep-wake cycle occurs in jetlag and shiftwork conditions and is associated with both obesity and insulin resistance, although whether this arises due to de-synchrony of behavioral and metabolic rhythms has not been established. Here, we demonstrate that genetic abrogation of the neural circadian system leads to the mis-timed feeding under entrained conditions that becomes arrhythmic in constant darkness. Hyperinsulinemic euglycemic clamp experiments in brain clock knockout mice (Camk-Cre::Bmal1, BKO) reveal increased hepatic glucose production that is resistant to suppression by insulin, in addition to reduced glucose disposal in adipose tissue and skeletal muscle. Correspondingly, the phase of glucose, insulin, and leptin is advanced in BKO mice, and the rhythmic transcription of metabolic networks within liver and adipose tissue is altered. Restricting food access in BKO mice to the subjective night reverses peripheral metabolic defects in these animals. Appetitive drive is likewise subjected to circadian regulation with markedly greater re-feeding in response to food deprivation at the beginning of the dark period, an effect that is associated with selective increases in the level of NPY/AgRP but not changes in POMC/CART. Chemogenetic acute and chronic silencing of pacemaker neurons similarly modulates activity behavior, feeding, and the respiratory exchange rate. These results reveal a hierarchical role of central pacemaker neurons in the synchrony of feeding time, energetics, and peripheral glucose metabolism, and indicate that forced food restriction can ameliorate adverse metabolic consequences of genetic de-synchrony between feeding behavior and the light-dark cycle.

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Functional elucidation of neural circuits interconnecting metabolic and circadian processes

Lora Heisler[1]

The phylogenetically ancient signaling molecule 5-HT (serotonin) is present in most species and orchestrates a diverse array of behavioral and physiological processes, interconnecting metabolic and circadian rhythms. Specifically, brain 5-HT has an inverse relationship with food intake and body weight; increasing 5-HT levels reduce food intake and body weight and decreasing 5-HT has the opposite effect. 5-HT appears to achieve these effects primarily via action at the 5-HT2C receptor subtype (5-HT2CR) within the homeostatic brain region, the arcuate nucleus of the hypothalamus. 5-HT also projects to the suprachiasmatic nucleus (SCN), a crucial nexus for the maintenance of circadian rhythms. 5-HT2CRs are also abundantly expressed in this brain region. Data is presented which connects metabolic and circadian processes via 5-HT action at 5-HT2CRs.

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Work funded by the Wellcome Trust and BBSRC.

Chemogenetic analysis of circuits involved in energy usage with implications for obesity treatment

PB Martinez de Morentin

Globally, the obesity epidemic has become one of the largest challenges to human health not only because of the comorbidities linked to it, such as type 2 diabetes, cardiovascular stroke and cancer, but also because its high morbidity rates. Understanding molecular underpinnings of energy balance (food intake and energy expenditure) is crucial to uncover new strategies to combat obesity. Historically, most obesity medications have targeted food intake. However, increasing energy expenditure has now become tractable with the identification of brown fat (BAT) in adult humans. BAT is a unique tissue capable of reducing fat excess by dissipating energy as heat. Thus, by turning on BAT, fat is burned off without the need for physical exercise and body weight is reduced. Since the

brain is the major manager of energy homeostasis, deciphering the neuronal networks driving this regulation is crucial to understand how new therapies can be developed.

Rowett Institute of Nutrition and Health, Aberdeen, UK

Selected Talks 2: Featured Speaker

X-linked histone demethylase Kdm6a regulates cerebellar development and motor coordination

Jun Xu[1], Halle Weimar[1], Terri Driessen[1], and Ge Kai[2]

Kdm6a is an X-linked histone demethylase that activates gene expression via removal of the repressive methylation mark at histone H3 lysine 27 (H3K27). In humans, *KDM6A* mutations cause Kabuki syndrome, a disorder characterized by intellectual disability and motor coordination deficits. To assess the role of Kdm6a in brain development and behavior, we generated a neuron-specific Kdm6a deficient mouse model (Kdm6aDef). The Kdm6aDef mice and wild type (WT) littermates have comparable body weight, reproductive behavior, and lifespan, different from the constitutive female Kdm6a KO mice which die prenatally. The Kdm6aDef and WT mice scored similarly in tests such as fear conditioning, locomotor activity, and grip strength. However, these mutant mice exhibited an adult-onset deficit in motor coordination. Gene expression analysis with RNA-seq and RT-qPCR identified mis-regulated genes in Kdm6aDef mice, including up-regulated expression of cerebellar granule cell-specific genes (e.g. *Zic1*, *Etv1*, *Gabra6*, and *Grin2c*). Some of these genes, *Zic1* and *Etv1* for instance, are known to be crucial to the proliferation and differentiation of granule cell precursors. We thus hypothesized that, in the granule cell precursors of Kdm6aDef mice, gene mis-regulation leads to enhanced proliferation of precursors, and subsequently cerebellar abnormality in the inhibition-excitation balance as well as behavioral impairment in motor coordination. We are currently testing this hypothesis with methodologies such as BrdU cell lineage analysis. We believe that these studies will shed light on the etiology of Kabuki syndrome as well as in general the epigenetic basis of brain development and motor behavior.

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Selected Talks 2: Speaker 2

Genomic and brain transcriptomic variation of mouse microRNAs

Kalevi Trontti[1], Juho Väänänen[1], Katherine Icay[2], Tessa Sipilä[2], Dario Greco[3], Iiris Hovatta[1,2,4]

MicroRNAs (miRNAs) are small non-coding RNAs that function in the post-transcriptional regulation of gene expression. The role of individual miRNAs in neurobiological functions has recently been revealed. However, it is not well understood how genetic variation within miRNA loci influence the function of miRNAs. We systematically investigated genetic variation within the miRNA genes in the mouse genome. We used publicly available whole genome sequence dataset of 36 inbred mouse strains (http://www.sanger.ac.uk/resources/mouse/genomes/). We also carried out miRNA and mRNA sequencing (miRNAseq and RNAseq) from frontal cortex and hippocampus of 6 laboratory strains to investigate the brain miRNA transcriptome. We observed genomic variation occurring less frequently within the miRNA loci compared to the rest of the genome. We found 719 and 779 miRNAs expressed in the frontal cortex and hippocampus, respectively, of which 226 were differentially expressed between the strains. Using two de novo miRNA predictions tools, we identified 105 putative novel mouse miRNAs. By comparing the DNA variation data with the miRNAseq data we found consistent RNA editing of the seed region in three miRNAs: miR-411-5p, miR-376b-3p, and 467d-5p. RNA editing and SNPs (N=29) in the seed region affected their predicted mRNA targets largely. Consequently, the biological pathways potentially regulated by the two miRNA alleles were different as revealed by the Ingenuity Pathways Analysis. To conclude, our comprehensive characterization of mouse miRNA genes and their expression patterns reveal inbred mouse strains as a genetic model system for investigating the effect of genomic variation within the miRNA genes on various behavioral phenotypes.

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Selected Talks 2: Speaker 3

An epigenetic link of acute inebriation to presynaptic changes and the development of alcohol tolerance, preference, and reward

Gregory L. Engel[1],Sunanda Marella[2], Karla R. Kaun[3], Julia Wu[2], PratikAdhikari[1], Eric C. Kong[2], Fred W. Wolf[1,2*]

Acute ethanol inebriation causes neuroadaptive changes in behavior that favor increased intake. Ethanol-induced alterations in gene expression, through epigenetic and other means, are likely to change cellular and neural circuit function. Ethanol markedly changes histone acetylation, and the sirtuin Sir2/SIRT1 that deacetylates histones and transcription factors is essential for the rewarding effects of chronic drug use. The molecular transformations

leading from acute to chronic ethanol responses mostly remain to be discovered. We find that Sir2 in the mushroom bodies of the fruit fly Drosophila promotes acute ethanol-induced behavioral plasticity by allowing changes in the expression of presynaptic molecules. Our findings tie the gene regulatory effects of acute ethanol to ethanol induced behavioral plasticity in a brain region that associates context with innate approach and avoidance responses. How these ethanol induced molecular changes impact neuronal function and also interface with other molecular and cellular effects of acute ethanol will help define how circuits change to reinforce drug intake.

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Selected Talks 2: Speaker 4

Genetic divergence in the engram for chronic alcohol exposure
M.K. Mulligan[1], K. Mozhui[1], A.K. Pandey[1], L. Lu[1], R.W. Williams[1]

Genetic factors that influence the transition from initial drinking to dependence remain enigmatic. Recent studies have leveraged chronic intermittent ethanol (CIE) paradigms (Becker and Lopez 2004, PMID 15608599) to measure changes in brain gene expression in a single strain at 0, 8, 72h (Osterndorff-Kahanek et al. 2015, PMID 25803291 and Melendez et al. 2012, PMID 21812870), and even 7 days following CIE (Smith et al 2016, PMID 26730594). Changes dwindle after 8h with few detected at 72h and 7d, although these may reflect long lasting alterations that underlie susceptibility or resistance to addiction. We extend these findings using LCM RNA-seq to profile expression in 11 brain regions in C57BL/6J (B6) and DBA/2J (D2) 72h following CIE. Linear models identified differential expression based on treatment, region, strain, or interactions. Nearly 60% of genes showed a robust effect (FDR < 0.01) of region and another 10% showed a robust effect of strain. Treatment had a smaller impact. Only 72 genes ($p < 0.01$) are differentially expressed due to treatment, although several (*Lyz2*, *Gpr17*, *Pcsk7*) overlap findings from previous studies and are likely to represent general response to CIE. Many more (415) demonstrate strain-specific responses. These are enriched in processes related to RNA metabolism and transcription factor activity (up in B6 and down in D2), and mitochondrial function (down in B6 and up in D2). Substantial strain differences exist in the temporal pattern of transcriptional neuroadaptation to CIE and these may drive individual differences in risk of addiction following excessive alcohol consumption.

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Support: INIA grant U01AA13499.

Selected Talks 2: Speaker 5

Pyruvate carboxylase functions in astrocytes to regulate habituation learning.

Jennings, C., Johnson, L., and Wolman MA.

Animals constantly update their behavior by evaluating the significance of current sensory input and then integrating this information with knowledge gained from prior experiences. To filter irrelevant input and focus attention towards high priority stimuli, all animals exploit a fundamental form of learning, called habituation. Habituation is observed by a progressive response decline to repeatedly experienced, yet insignificant stimuli and provides a behavioral measure of a neural circuit's ability to balance synaptic excitation, inhibition, and plasticity. Habituation deficits are observed in schizophrenia, autism, and addiction, and contribute strongly to the patients' overall dysfunction. Despite its conservation and clinical relevance, the genetic and cellular mechanisms that mediate habituation remain poorly understood.

To identify genes critical for habituation, we recently performed an unbiased, genome-wide screen for zebrafish mutants with reduced habituation of the acoustic startle response. This screen identified a functional gene set for habituation, including a gene previously unknown for a role in habituation: *pyruvate carboxylase*. PC is a mitochondrial enzyme that converts pyruvate to oxaloacetate to stimulate the TCA cycle, and hence, the synthesis of metabolic products with diverse biological functions. In the brain, PC is not thought to be active in neurons, but instead, functions in oligodendrocytes to promote myelination and in astrocytes to replenish neurons with neurotransmitters. Notably, neither cell type is known to regulate habituation.

Through molecular-genetic, pharmacological, and behavioral analyses, we will provide data showing that astrocytic PC activity supports glutamatergic signaling to promote habituation. This work defines a novel molecular mechanism underlying habituation and reveals a novel cellular locus of plasticity for habituation: astrocytes!

Department of Zoology, University of Wisconsin, Madison.

Poster Session 2 (Even Numbers)

2. Early behavioral markers of schizophrenia in DISC1 (Disrupted-in-Schizophrenia-1) knockout rats

MJ Glenn[1], W Yu[1], AA Batallan[1], KD Riley[1],JA Honeycutt[2], JR Mitchell[1]

The DISC1 gene contributes to the development and function of the central nervous system and alterations in it are linked to psychiatric disorders, particularly schizophrenia. In humans, DISC1 translocation affects brain areas dysfunctional in schizophrenia (hippocampus, frontal cortex); at the subcellular level (centrosome, mitochondria) the gene product is important in neuron migration, branching, and signal transduction. A new DISC1 disruption model in

rats is poised to add substantively to our understanding of gene-behavior links by building on decades of rat neurobehavioral research. Supporting the validity of this model to reproduce schizophrenia-like symptoms, our lab recently found that adult Sprague-Dawley DISC1 knockout rats, compared to wildtypes, exhibited increased locomotor activity and anxiety-like behavior. At present, we continue to investigate the behavioral effects of the knockout and aim to uncover underlying neural mechanisms. In this study, we compared weanling and adolescent female and male DISC1 knockout rats to age-matched wildtypes to address 3 goals: 1) to detect potential early markers in the behavior of DISC1 knockout rats comparable to the subtle behavioral and intellectual abnormalities observed in otherwise healthy human adolescents who later develop schizophrenia; 2) to search for biological sex differences in symptom kind, emergence, and severity; and 3) to examine neural changes that may lead to these effects. Our results so far point to exploratory, social, and information processing deficits that emerge at weaning with some sex differences in timing and magnitude. We are examining whether these may arise from problems with hippocampal plasticity and prefrontal-cortical inhibitory interneurons.

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Support: NIH NCRR 5P20RR016463-12 NIH NIGMS 8P20GM103423-12 (USA)

4. Congenital Muscular Dystrophy with Megaconial Myopathy (MDCMC): When big isn't better.
Sayed AA[1,2]., Cox GA[2], Sher RB[1].

Congenital muscular dystrophies (CMDs) include over 30 types of muscle disorders, all having an onset at or just after birth. Most CMDs are autosomal recessive disorders with rapidly progressing symptoms that have clinical manifestations resulting in muscle weakness and resulting delayed/arrested motor abilities. Many of these disorders include respiratory, cardiac illnesses and delayed speech development. Through our project, we plan to concentrate on Congenital Muscular Dystrophy with Megaconial Myopathy (MDCMC), a rare type of CMD, in which patients manifest muscular dystrophy in a rostral to caudal gradient with enlarged mitochondria localized at the cellular periphery and severe cognitive impairments. MDCMC has been shown to arise as a result of mutation in the Choline Kinase beta (*CHKB*) gene that codes for the enzyme choline kinase beta, which helps in phosphorylating choline to phosphocholine in the Kennedy pathway in skeletal muscles. We have discovered a mouse with a 1.6kb deletion in the choline kinase beta gene, which showed a phenotype as described above, making it an excellent model for the study of MDCMC. Since, then we have engineered a Transgenic rescue mouse by injecting mouse *Chkb* gene under the control of titin promoter. In this project we have characterized this rescue mouse and also tested if the rescue's can be a good model for cognitive impairments as seen in the human patients.

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6. Long-term consequences of chronic-intermittent ethanol vapor exposure on affective behavior in selectively-bred Withdrawal Seizure Prone and Withdrawal Seizure Resistant mice

AM Rosenwasser[1,2,3], WD McCulley III[1], M Hartmann[3], SE Holbrook[2], JC Crabbe[4]

Ethanol withdrawal is associated with both short- and long-term physiological and behavioral consequences. While much is known regarding the expression of acute withdrawal symptoms, less is known concerning long-term consequences in post-dependent individuals. Since relapse to excessive drinking commonly occurs long after initial detoxification, it is critical to understand the affective disruptions that sometimes persist during protracted abstinence, and that may be linked to increased risk of relapse. In the present study, we explore the long-term consequences of chronic-intermittent exposure to ethanol vapor (CIE) in a well-established animal model. Withdrawal Seizure Prone (WSP) and Withdrawal Seizure Resistant (WSR) mice were selectively bred for high and low levels of handling-induced convulsions following withdrawal from CIE. Little is known, however, about possible enduring behavioral disruptions that might occur in these lines. In the present study, male and female WSP and WSR mice from the second selection replicate (WSP2, WSR2) were exposed to CIE and then subjected to several behavioral tests at weekly intervals for six weeks. Behavioral measures included the sucrose preference test, a well-established test for depression-like anhedonia, the light-dark box test, a commonly used index of anxiety-like behavior, and the marble burying test, thought to reflect a form of anxious-compulsive-like behavior. While testing is still underway, initial results indicate that CIE mice display increased sucrose preference and increased marble-burying for about 2 weeks post-CIE, relative to air-exposed controls. Thus far, no clear genotypic differences have emerged, suggesting that acute and chronic withdrawal signs may be associated with different underlying genetics.

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8. Altered Energy Balance in Ethanol-Treated Animals

M. Blaszkiewicz[1], M. Hartmann[1], C. Curtis[2], A. Rosenwasser[1], K. Townsend[1,2]

Ethanol is a common part of the human diet. However, very little is known about the effects of ethanol intake on various aspects of energy balance. Nevertheless, it is well known that ethanol intake disrupts circadian rhythmicity, and that proper circadian regulation is essential for maintenance of energy homeostasis and metabolism. We propose that ethanol intake in our rodent model interferes with normal energy balance and promotes a reversible effect on fuel utilization and

metabolism, in part through changes in gene expression in key metabolic tissues, such as Rev-erbB in the hypothalamus. We have shown that C57BL/6J mice (an obesity-prone strain characterized by high voluntary ethanol intake) consuming 10% EtOH over just several days, display decreased VCO2 and decreased RER, the latter indicating greater utilization of fatty acids as fuel. These trends completely reverse upon cessation of EtOH. Furthermore, these changes occur without any differences in food intake or total caloric intake and are accompanied by a trend for decreased weight gain and decreased subcutaneous white adipose mass in EtOH mice, together demonstrating that ethanol intake may be protective against increased adiposity by favoring fatty acid utilization as fuel. Along these lines, female mice fed a high-fat diet along with 10% EtOH treatment display a striking improvement in glucose sensitivity versus control animals on a high fat diet. For mechanistic insight, we have implicated changes in gene expression in hypothalamus, liver, and adipose tissues and are working to determine the molecular pathways involved in the observed phenotype.

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10. Dopamine signaling in health and disease, pathways and related networks at RGD's Pathway Portal

V Petri[1,2], GT Hayman[1,2], JR Smith[1,2], M Tutaj[1,2], J de Pons[1,2], MR Dwinell[2,3], M Shimoyama[1,2], the RGD Team[1,2]

Dopamine (DA) acts as a neurotransmitter and neuromodulator in the nervous system and as an autocrine/paracrine hormone peripherally. In the brain DA is involved in motor control and action selection, working memory and behavior, cognition and reward functions, and more. These roles are achieved through the networks DA neurons establish. DA neurons, mostly in midbrain regions, project to the striatum, the frontal cortex and other regions to form specific neural pathways. DA signaling employs five receptors – G- protein coupled receptors (GPCRs), subdivided into two subtypes coupling to distinct G alpha subunits (a heteromer, a third). As such, dopamine prompts distinct intracellular cascades and downstream events. Changes, alterations or loss of dopamine input are associated with various conditions. Loss of DA neurons in substantia nigra pars compacta (SNc) is the hallmark of Parkinson's Disease (PD); changes in other DA systems are involved in mental disorders and addiction. PD is largely sporadic and age is a major risk factors. Ageing affects mitochondria homeostasis and epigenetic changes; defects in several genes impact on these functions and relate to PD. Interactive diagram pages for dopamine signaling and Parkinson disease pathways are part of molecular pathways collection at Rat Genome Database (RGD) Pathway Portal. Pathways pertinent to mitochondria function and epigenetic changes are also present. Directly connected pathways are displayed within a diagram page; pathways revolving around a broader concept are brought together in pathway suites and suite networks. Mitochondria

homeostasis is a suite example; epigenetics is one of the suites within a suite network.

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Support: National Heart, Lung, and Blood Institute on behalf of the NIH (USA), HL64541

12. Examination of cerebellar development across time in C57BL/6J, DBA/2J and BXD mice
KM Hamre[1], E Brauer[1], D Swanson[2], T Ha[2], D Goldowitz[2]

Previous data have shown differences in cerebellar size and morphology in adult animals of differing genetic backgrounds. However, the morphologic mechanisms and molecular pathways that cause these differences remain unknown. To address these issues, BXD recombinant inbred mice and parental C57BL/6J and DBA/2J inbred strains were examined. Mice were examined across developmental ages starting at embryonic day 12.5, near the beginning of cerebellar development, and continuing into postnatal days and to adulthood. Measures examined included: numbers of different cell types and cell density, cerebellar volume and volume of different layers. Adult mice were examined to assess the relationship between numbers of various cell populations and the regional volumes, and to examine QTLs (quantitative trait loci) for these differences. In these analyses, we demonstrate that there were strong correlations among many of the measures examined suggesting that genetic differences influence cerebellar development as a whole rather than differentially affecting certain populations. We also identified a novel QTL on mid-murine chromosome 14 controlling differences in Purkinje cell number. Analyses across time showed strain-specific patterns of development and that, in general, the strains that were larger on most measures at the beginning of cerebellar development were also the strains that were largest later in development. This suggests that early developmental events were major contributors to cerebellar size and cell number. Using the database cbgrits.org we have captured the expression profiles of B6, D2, and 14 BXD RI lines in CbGRiTS.org and will present gene-structure correlations.

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Support: R01HD052472 and NeuroDevNet.

14. NICOTINIC ACETYLCHOLINE RECEPTORS INFLUENCE ETHANOL BEAHVIORS IN AN AGE SPECIFIC MANNER
H.M. Kamens, C. Peck, & A. Gechlik

Alcohol and nicotine are both commonly used drugs, and are often co-abused. In both humans and animal models, genetic correlations have been reported between the responses to these drugs. These studies indicate that common neural mechanisms underlie alcohol and nicotine behaviors. Nicotine binds to nicotinic acetylcholine receptors where it has physiological actions. Moreover prior research has also implicated these receptors in alcohol responses. In many cases, alcohol use begins in adolescence, when the brain is undergoing structural and functional changes. Important for this work, nicotinic acetylcholine receptors continue to change during this period of development. To assess the role of nicotinic acetylcholine receptors in adolescent ethanol responses we used varenicline, a partial agonist with high affinity for α4β2 receptors. Adolescent C57BL/6J mice were tested for alcohol behaviors by observing ethanol consumption, ataxia, and sedation. These behaviors were chosen because research in adult animals has provided evidence of the involvement of α4β2 nicotinic acetylcholine receptors in these responses. In adult C57BL/6J mice, varenicline decreases ethanol consumption without modulating water or saccharin intake. However, in adolescent animals, varenicline decreased ethanol consumption and sucrose intake revealing the effect was not specific. Moreover, in adult animals varenicline increased ethanol-induced ataxia and sedation. In contrast, in adolescent C57BL/6J animals, varenicline had no effect on ethanol-induced motor incoordination or sedation. These data provide evidence of age-specific effects of the involvement of nicotinic acetylcholine receptors in ethanol behaviors.

The Department of Biobehavioral Health, The Pennsylvania State University, University Park, PA
Support: AA019447 and the College of Health & Human Development. Varenicline was generously provided by Pfizer.

16. The genetic architecture of open-field behavior in mice
Anna Delprato[1,2,3], Marie-Paule Algéo[1,2], Brice Bonheur[1,2], Lu Lu[4], Robert W. Williams[4], Wim E. Crusio[1,2]

The open field is a classic test used to assess exploratory behavior, anxiety, and locomotor activity in rodents. In this study we used 53 BXD recombinant inbred mouse strains to map quantitative trait loci (QTLs) underlying behaviors displayed in an open field. For most strains, we had sample sizes of 10 male and 10 female mice. The traits assessed during a session of 20 min were percent time and distance spent near the wall (thigmotaxis), leaning against the wall, rearing, jumping, grooming duration, grooming frequency, locomotion, and defecation. All of these traits were known to exhibit moderate heritability. We identified a significant QTL on chromosome 4 that influences grooming duration in both males and females. A suggestive QTL that modulates locomotion also maps to this same region and others have reported a significant QTL for locomotor activity mapping to the same locus. Based on the selection criteria and

published literature associating *Disabled-1* (*Dab1*, part of the reelin signaling pathway) with grooming and locomotor activity, we propose *Dab1* as the candidate gene influencing both behaviors. We also identified a pairwise epistatic interaction between loci on chromosomes 12 and 14 that influences rearing frequency in males. Here, too, previous studies provide supporting evidence for the chromosome 12 locus.

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18. Detecting interactions between *Gtf2i* and *Gtf2ird1* in mouse behavior and oxytocin regulation

Nathan D. Kopp[1] and Joseph D. Dougherty[1,2]

William's syndrome is a neurodevelopmental disorder with a prevalence of 1/7000 births. It is caused by a microdeletion of 1.8 Mbp on chromosome 7q11.23, which results in cardiovascular, craniofacial, visual-spatial, and social aberrations. Further, human studies of patients with William's syndrome have discovered that there is an increase in plasma oxytocin levels compared with typically developing controls. Two general transcription factor 2I genes, *GTF2I* and *GTF2IRD1*, are commonly deleted and are high priority candidates for mediating the hypersocial phenotype in humans and mouse model. However, it is not clear if they act independently or collaboratively to produce the phenotype. We have used the CRISPR/Cas9 system to delete these transcription factors in various combinations in mice to investigate how the interaction of these genes affect the social phenotype and understand possible molecular intermediaries that manifest the hypersocial behavior. We have conducted longitudinal behavioral phenotyping on the offspring of a *Gtf2i*$^{+/-}$ and *Gtf2ird1*$^{+/-}$ cross, so that we can compare the social behaviors and cognitive abilities of all four possible genotypes. Finally, to reconcile the human observation of increased oxytocin and previous mouse behavior data, we investigate the plasma oxytocin levels of *Gtf2i*$^{+/-}$, *Gtf2ird1*$^{+/-}$, *Gtf2i*$^{+/-}$*/Gtf2ird1*$^{+/-}$, and mice with the complete Williams syndrome critical region deleted in cis (CD).

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20. Modeling increased autism risk following maternal SSRI use.

SE Maloney[1,2], S Akula, [1,2], K McCullough[1,2], K Chandler[1,2], JD Dougherty[1,2]

Recent epidemiology studies found a significant association between maternal antidepressant use and increased risk of autism in offspring, independent of mothers' depressive symptoms. These human studies necessitate explanatory studies in model organisms to elucidate consequences on behavior of maternal SSRI use, and identify mechanisms. The objective of this study was to model the recent human patient population findings to directly examine the risk of presenting autism-relevant behaviors following developmental SSRI exposure. Clearly, not every mother taking an SSRI has a child with autism, suggesting there are other factors mediating the risk of developing autism following SSRI exposure. Another source of altered serotonin activity during brain development is mutation and polymorphism in genes important for the functioning of serotonin neurons. Mouse models null for such genes, including serotonin cell-enriched proteins like Celf6, exhibit autism-related behaviors. Furthermore, polymorphisms in *CELF6* have been associated with ASD risk in humans. This suggests genetic variation in human serotonin-related genes may contribute to the impact of SSRI exposure on autism risk. Therefore, I also examined the interaction of environmental SSRI exposure and genetic susceptibility to autism risk in the *Celf6* mutant line. Female mice were treated with the SSRI fluoxetine (FLX), equivalent to the maximum recommended human dose, from prior to pregnancy through lactation. FLX- and vehicle-exposed offspring, beginning postnatal and continuing through adulthood, were evaluated for social and communication behaviors, sensorimotor and locomotor abilities, and repetitive patterns of behavior. While no interaction was observed with Celf6 genotype, FLX-exposed mice displayed substantial deficits in social communication, social dominance and repetitive behaviors.

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22. Region-Specific $A\beta_{1-42}$ and TXNIP expression in the aging hippocampus of 3x-TgAD and control (non-Tg) mice

Wheeler, Ryan V[1]; Goguen, Donna[1]; Brown, Richard E[1]; Weaver Ian CG[1]

Aging, inflammation, cardiovascular disease and diabetes are the major non-genetic risk factors for Alzheimer's disease (AD). Studies in human AD and 5xFAD transgenic (Tg) mouse models, have shown that the toxic 42-mer Aβ ($A\beta_{1-42}$) oligomer implicated in AD pathology can trigger the expression of the pro-apoptotic factor, thioredoxin interacting protein (TXNIP), which stimulates pro-inflammatory cytokines expression, resulting in reduced glucose metabolism, insulin resistance, oxidative stress and neuronal loss (Gouget et al., Alzheimer's Dement. 7, S684, 2011). This suggests TXNIP potentially plays an important role in mediating the $A\beta_{1-42}$ oligomer's detrimental action on neuronal degeneration, which may underlie the spatial memory deficits observed in the 3x-TgAD mice

(Stover et al., *Behav. Brain Res.* **289,** 29–38, 2015). This current study elucidates the hippocampal regional expression of TXNIP in 17 month old females of the 3x-TgAD mouse model of AD using immunofluorescence. In addition, the colocalization of TXNIP and $A\beta_{1-42}$, is examined to display the relationship between this toxic oligomer and TXNIP. Overall, a distinctive regional-functional relationship is observed within the localization and quantity of TXNIP in the CA1-3 regions of the hippocampus between the transgenic 3x-TgAD mice and the wild-type controls (B6129SF2) suggesting the potential role of TXNIP in AD-related pathology and subsequent symptomology.

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24. A Novel Gene for Amyotrophic Lateral Sclerosis Identified

Martin, PB[1,2], Sher, RB[1,3], Cox, GA[1,2]

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder that affects primarily motor neurons in the spinal cord, brainstem, and motor cortex. Degeneration of the motor neurons leads to progressive paralysis, atrophy of denervated muscles, and ultimately death, with a median survival of 2-5 years. We have identified two independent ENU-induced mouse mutations in the nuclear export mediator factor (*Nemf*), which cause motor neuron disease. The mutant mice exhibit phenotypic hallmarks of human ALS, including loss of hind limb function, loss of peripheral motor and ventral root axons and neuromuscular junction denervation. As well as, nucleo/cytoplasmic mislocalization of the ALS marker TAR DNA binding protein (TDP43), and increased nuclear ubiquitination of proteins in spinal motor neurons. *Nemf* (Rqc2/Tae2 in yeast) is known to function as a subunit of the Ribosome-Associated Protein Quality Control (RQC) complex together with the E3 ligase Listerin, whose mutation in mice has been reported to phenocopy the *Nemf* ENU-idnduced mutations we have discovered. We continue to search for mutations in the human *Nemf* gene in amyotrophic lateral sclerosis and other neuromuscular disease patients.

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26. Mouse Phenome Database: Enhancing experimental protocols to address research reproducibility and replicability

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Inadequate reporting reduces the impact of research and hampers effective translation of scientific discoveries from basic/preclinical findings to human applications. Substantial recent attention has been given to poor cross-species relevance, replicability and reproducibility of model-organism behavioral studies. The reasons for poor reproducibility are many-fold and include, underpowered studies, poorly documented experimental protocols and lack of adequate

identification of research resources such as mouse strains and reagents. ARRIVE and other guidelines for scientific reporting have been developed to ensure that studies are reported with detailed information to allow researchers to evaluate results, repeat experiments and extend findings. The Mouse Phenome Database (MPD; phenome.jax.org) is a widely used online resource providing access to primary experimental data, protocols and analysis tools. Data come from investigators around the world and represent a broad scope of behavioral endpoints and disease-related characteristics in naïve mice and those exposed to drugs, environmental agents or other treatments. MPD provides an important venue for compliance with data sharing policies and facilitates data reuse and data integration to provide a means of assessing replicability and reproducibility across experimental conditions and protocols. MPD has consistently provided rigorous curation of mouse experimental data and supporting documentation. Restructuring protocols and providing additional information now required by NIH and leading scientific journals will help ensure reproducibility. This will better enable investigators to interpret results from different behavioral assays and benchmark or replicate experimental data.

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28. The effect of nicotine exposure on ethanol consumption and gene expression: a WGCNA analysis

Silva, C.P.[1], Caruso, M.J.[1, 2], Horton, W.J.[2, 3] & Kamens, H.M[1, 2].

Nicotine, the principal addictive component of tobacco, and alcohol are both highly co-abused substances. The aim of this study was to determine how exposure to nicotine during adolescence alters ethanol intake and resulting gene expression. In the first phase of the experiment, adolescent female C57BL/6J mice were exposed to water or 200 µg/ml nicotine in drinking water for 22h and to a single bottle of water for 2h a day, for six days. Following this period, these two groups were further divided into ethanol or control groups, resulting in four total conditions. In this second phase, the 4 day drinking-in-the-dark (DID) paradigm was overlaid during the 2h when mice did not have nicotine available. Thus, mice were exposed to water or nicotine for 22h and to a single tube filled with 20% v/v ethanol or water for 2h (or 4h on the last day). Following the final DID session, whole brains were collected for RNA sequencing. Resulting normalized gene expression (FPKM) were analyzed and signed networks were built using Weighted Gene Co-expression Network Analysis (WGCNA). Adolescent nicotine increased ethanol consumption and blood ethanol concentrations. Moreover, three significantly different gene co-expression modules were identified between the nicotine-ethanol group and water-water controls. Hub genes within these modules, were associated with mRNA maturation, transport and splicing (Hnrnpa3 and Nup54), synapse formation (Nlgn2, Bad, Ccnf, Ptk7 and Gan), and synaptic assembly and neuropeptide hormone activity (Lrrc4b, Nrxn2 and Oxt). These results suggest that nicotine-

ethanol treatment altered RNA expression of genes involved in adolescent brain development.

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Support: K01 AA019447 and the Huck institute of the Life Sciences

30. Voluntary wheel-running affects anxiety behavior and physiology in mice fed a high fat diet
NL Arruda, JA Hicks, RR Gelineau, I De Pina Monteiro, A Hatzidis, JA Seggio

It is widely accepted that lifestyle plays a crucial role on the quality of life in individuals, particularly in western societies where poor diet is correlated to alterations in behavior and the increased possibility of developing type-2 diabetes. While exercising is known to produce improvements to overall health, there is conflicting evidence on how much of an effect exercise has staving off the development of type-2 diabetes or counteracting the effects of diet on anxiety. Thus, this study investigated the effects of voluntary wheel-running access on the progression of diabetes-like symptoms and open-field and light-dark box behaviors in C57BL/6J mice fed a high-fat diet. C57BL/6J mice were placed into either running-wheel cages or cages without a running-wheel, given either regular chow or a high-fat diet, and their body mass, food consumption, glucose tolerance, insulin and c-peptide levels were measured. Mice were also exposed to the open-field and light-dark box tests for anxiety-like behaviors. Access to a running-wheel partially attenuated the obesity and hyperinsulinemia associated with high-fat diet consumption in these mice, but did not affect glucose tolerance or c-peptide levels. Wheel-running strongly increased anxiety-like and decreased explorative-like behaviors in the open field and light-dark box, while high-fat diet consumption produced smaller increases in anxiety. These results suggest that voluntary wheel-running can assuage some, but not all, of the physiological problems associated with high-fat diet consumption, and can modify anxiety-like behaviors regardless of diet consumed.

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32. Severity of Demyelinating and Axonal Neuropathies Are Modified by Genetic Mutations Affecting Sodium Channels at Nodes of Ranvier
KH Morelli[1, 2] KS Seburn[2], DG Schroeder[2], GA Cox[2], RW Burgess[1, 2]

Charcot-Marie-Tooth disease (CMT) is a collection of inherited neuropathies caused by either nerve demyelination or axonal dysfunction. The typical clinical presentation of CMT includes muscle weakness and atrophy, although the severity of each case varies greatly. Recently, we identified a mouse strain that

developed progressive weakness and hind limb dysfunction. Genetic mapping and exome sequencing revealed a double null mutation in *Sh3tc2*, a gene linked to a demyelinating CMT (CMT4C) and *Nrcam,* a gene that encodes a protein involved in sodium channel localization at nodes of Ranvier. The phenotype of *Sh3tc2* mutant mice closely resembles that of reported mouse models of CMT4C, whereas mice lacking NRCAM have only a subtle reduction in nerve conduction velocity. In combination, these mutations result in paralysis and severe deficits in neuromuscular junction innervation. Thus, we hypothesized that subclinical deficits in sodium channel function at nodes synergize with mutations affecting the passive propagation of depolarization to cause a more severe phenotype. We tested this hypothesis by breeding the *Nrcam* mutation onto a *Gars* mutant background, a model of an axonal CMT that compromises the axonal length constant by reducing axon diameter. Interestingly, the loss of NRCAM also exacerbated the *Gars* phenotype. We validated that the defect is due to impaired sodium currents by showing that *Scn8a* heterozygotes, mice that have a 50% reduction in sodium channels at nodes, phenocopy *Nrcam* null mice. These data demonstrate that genes encoding node proteins are potential modifier loci for CMT and may account for its variable severity among patients.

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34. Short-term selective breeding for adolescent sensitivity to tetrahydrocannabinol- (THC-) induced locomotor sedation in mice.
SL Boehm II, Y Zhang, CR Kasten

Although the endocannabinoid system undergoes considerable developmental change during adolescence, cannabis use during this time is a growing problem in the US (Volkow et al., 2014). The goals of the current project are to determine whether genotype influences adolescent locomotor sensitivity to the sedative effects of tetrahydrocannabinol (THC; psychoactive component of cannabis), and whether genes that influence such sensitivity also influence other adolescent (and adult) phenotypes. Using a short-term selective breeding strategy originating from a B6D2F2 founding population (created by crossing C57BL/6J dams with DBA/2J sires), we have initiated the production of mouse lines exhibiting high and low adolescent sensitivity to THC. Adolescent (postnatal day 28-32) F2 males and females were assessed for locomotor sensitivity to a 10mg/kg THC dose, and mice exhibiting high and low sensitivity to the sedative effects were chosen for subsequent breeding of selection generation 1 (S1). The phenotyping of S1 offspring indicate the initial response to selection was stronger in the direction of low adolescent sensitivity to THC's locomotor sedative effects, with realized heritability (h^2) calculated at .05 and .91 for the high and low sensitivity lines, respectively. Thus, early results suggest considerable genetic influence on resistance to adolescent THC-induced locomotor sedation. Ongoing efforts are aimed at continuing selection beyond S1, as well as determining

whether genes influencing high adolescent sensitivity to THC-induced locomotor sedation also influence adult locomotor sensitivity to THC.

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Support: The current work is supported by grants from the IU Vice President for Research and the NIH-funded Indiana Clinical and Translational Sciences Institute.

36. Computer Vision Based Analysis of Complex Mouse Behaviors
Brian Geuther[1], Mayank Kabra[2], Kristin Branson[2], Vivek Kumar[1]

Endophenotypic behavioral measures are often used to quantify an animal's emotional state. The open field assay, first developed by Calvin Hall in the 1930s, is a classic assay that has been used to access hyperactivity and anxiety phenotypes. While current video based technology is adept at identifying location and velocity of animals, there is a wealth of information that cannot be properly analyzed due to lack of good automated scoring systems. Here we used JAABA, a machine learning and computer vision based system, to train classifiers for six behaviors. Our trained classifiers can detect grooming, rearing, jumping, scratching with high accuracy and performed as well or better than human scoring. We are currently applying our classifiers to data from segregating genetic crosses, inbred strains and disease models. Our goal is to link these complex behaviors to genetic loci and diseases.

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38. Are male mice better test subjects for behavioral phenotyping?
Fritz AK[1,2], Wolfer DP[1,2]

Many researchers still prefer to use only males for the behavioral screening of mice, typically stating that female mice perform more variably due to their estrus cycle. Male mice are sometimes also said to perform better in spatial tasks. Even though some studies seem to support these claims, unequivocal evidence is lacking. We addressed the question by a retrospective analysis of 5550 mice without hormonal treatment which had performed the same place navigation protocol in the water-maze and of 4850 mice tested in the same open field arena. Water-maze data comprised 550 experimental and control groups containing both male and female subjects. We found significant sex effects in favor of males in most training and probe trial measures, but on average they explained less than 1% of the variance in the data. Open field data from 480 mixed groups revealed that females were overall more explorative and deposited less fecal boli, but again effect sizes were very small. Comparison of standard deviations between female and male subgroups revealed no consistent difference. While variability was slightly higher in females for some measures of place navigation

acquisition and open field behavior, the opposite was seen in many measures of spatial retention. In conclusion, we cannot confirm the notion that male mice generally yield more reliable data. Sex differences in water-maze performance and open field behavior were present in a very large population, but excessively small. Our data do not support the recommendation to use only male mice for behavioral phenotyping.

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40. ApoE Isoform- and Sex-dependent Effects of Methamphetamine Exposure in Chronic Variable Stressed Mice

Eileen Ruth S Torres[1], Lance A Johnson[1], Sydney Weber[1], Jacob Raber[1,2].

Post-Traumatic Stress Disorder (PTSD) is a significant mental health disorder with a lifetime prevalence rate of 6.8% in US adults. Although significant trauma is required for diagnosis, not everyone that experiences trauma develops PTSD. Furthermore, women present an altered phenotype compared to men. Thus, genetic factors may influence the neuropathology and phenotype of PTSD. Nearly half of those with PTSD develop substance use disorder, with one commonly abused drug being methamphetamine (MA). Apolipoprotein E (apoE) is a lipid-binding protein involved in cholesterol metabolism and exists in 3 isoforms in humans: E2, E3, and E4. We have recently shown E2 is associated with enhanced severity of behavioral, cognitive, and neuroendocrine changes due to PTSD in both humans and mice expressing human apoE. We now aim to determine if apoE isoform alters how the brain copes with methamphetamine exposure after PTSD induction. We use young female and male mice expressing either E2 or E3 through targeted gene replacement. Animals undergo chronic variable stress then receive daily MA or saline injections. During MA exposure, behavioral and cognitive performance are assessed. Female MA-treated E2 mice demonstrate depressive-like behavior but appear more resilient to anxiety-like behavior compared to male MA-treated E2 mice. MA-treated E2 mice show poorer novel object recognition compared to controls. Notably, E3 mice do not show these MA-induced behavioral alterations. Thus, we show apoE isoform- and sex-dependent effects of MA exposure in chronically variably stressed mice. These effects warrant further studies and are important to consider for developing potential personalized therapeutic targets.

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42. Metabolic effects of menthol on WFS1-deficient mice

M Ehrlich[1], M Ivask[1], A Raasmaja[2,3], S Kõks[1]

Previous experiments of RNA sequencing showed that *Trpm8* gene was upregulated in the hippocampus of WFS1-deficient mice compared to wild-type littermates. TRPM8 is activated by cold (8-28^{o}C) and chemicals, e.g. menthol, which induce cold sensation. TRP ion channels are used as primary transducers of thermal stimuli for thermosensation. The aim of this study was to compare the metabolic differences and dose-response effects to menthol between WFS1-deficient mice and their wild-type littermates. Experiments were performed with 9-12 months old male F2 hybrids (129S6/SvEvTac x 129S6/SvEvTac). Five to twelve WFS1-deficient mice and wild-type mice were used in the study. Different menthol doses were administrated by oral gavage and metabolic effect was measured with metabolic cages (TSE Phenomaster). The life span of WFS1-deficient and wild-type mice was investigated. The results of measurements showed that WFS1-deficient mice have significantly higher energy metabolism and shorter life span compared to WT mice and specific menthol doses had a significantly higher reaction on the energy metabolism of WFS1-deficient mice compared to WT mice.
In conclusion, our results show that the shorter life span, metabolic changes and upregulation of *Trpm8* gene in WFS1-deficient mice suggest that WFS1-deficient mice have serious metabolic dysfunctions. Menthol has an opposite effect on the WFS1-deficient mice metabolic parameters by increasing them compared to wild-type mice.

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Support: Istitutional research funding IUT20-46 of the Estonian Ministry of Education and Research.

44. Test for allelic interaction of a QTL influencing methamphetamine sensitivity using a 112 kb congenic line crossed to gene-edited knockout lines for *Hnrnph1* and *Rufy1*

R. Keith Babbs[1], Neema Yazdani[1,2], Lisa Goldberg[1,2], Benjamin Chew[1], Kimberly Luttik[1], Camron D. Bryant[1]

We used congenic mouse lines spanning a region of chromosome 11 (50-60 Mb) to fine map a QTL for sensitivity to methamphetamine (MA)-induced locomotor activity. Using mice containing donor DBA/2J (D2) chromosomal regions on a B6 background, we identified a 206 kb QTL interval that was *necessary* for reduced MA sensitivity (D2<B6). This interval contains two protein-coding genes – *Hnrnph1* and *Rufy1*. To determine if this interval was *sufficient* to reduce MA sensitivity, we generated a 112 kb congenic containing only *Hnrnph1* and *Rufy1*. Furthermore, in order to implicate *Hnrnph1* or *Rufy1* as a quantitative trait gene (QTG), we are currently testing for an allelic interaction on the behavioral phenotype following inheritance of congenic and TALENs-edited null alleles. The results thus far demonstrate that inheritance of the 112 kb congenic region is not

only *necessary*, but also *sufficient* to cause a significant decrease in MA sensitivity. However, in contrast to the dominant mode of inheritance of the QTL from the larger 10 Mb congenic, here, the mode of inheritance was recessive, requiring two copies of the D2 allele. Additionally, the effect size was reduced somewhat from r=0.34 to r=0.29. These observations indicate that additional alleles outside of the 112 kb region contribute to the overall 10 Mb QTL signal. Allelic interaction will determine if one or both genes act as QTGs to modulate MA sensitivity. Finally, we are triangulating on the striatal transcriptome of the congenics versus knockouts to converge on likely molecular mechanisms that bridge genetic variation with behavior.

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Support: K99/R00DA029635 (C.D.B.), R01DA039168 (C.D.B), T32GM008541, TTPAS Burroughs Wellcome Fund (N.Y.)

46. Pharmacological dissection of hyperactivity and related behaviors in a subset of KOMP lines.

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Activity patterns, such as locomotor hyperactivity, light aversion, and exploratory drive, are important phenotypes in animal models of psychiatric disorders including ADHD, autism, and depression. Neurochemical systems such as dopaminergic, serotonergic, and adrenergic are genetically complex and are known to regulate these behaviors. The Knockout Mouse Project (KOMP2) at The Jackson Laboratory contains a battery of behavioral assays designed to assess endophenotypic traits of psychiatric disorders. These assays include open-field, light-dark, exploratory holeboard, acoustic startle response, tail suspension, and rotarod. Here, we evaluate a subset of lines that exhibit hyperactivity in the KOMP2 pipeline and attempt to pharmacologically dissect the neurochemical pathways contributing to the phenotype. Mutant and control mice are treated with drugs that target specific neurobiological pathways in order to observe their effect on hyperactivity in the open field. We present results with six pharmacological treatments that target dopaminergic, serotoninergic, glutamatergic, and GABAergic signaling in these mutant lines. These results provide enhanced characterization of new potential models of psychiatric disorders that target different neurobiological systems.

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48. An Emerging Role for Developmental Regulator Tcf7l2 in Social Learning and Behavior

Christopher Seward[1,2], Michael Saul[1], Joseph Troy[3], Patricia A. Weisner[4], Derek Caetano-Anolles[2], Huimin Zhang[1], Hao Sun[1], Lisa Stubbs[1,2,4]

Previous genomic studies in our laboratory have identified a conserved toolkit of transcription factors that respond to social threat in three diverse species, mice, honeybees, and stickleback fish. These studies identified WNT signaling as one of the most consistent and dominant signals. In mice, Tcf7l2, which encodes an ultimate effector TF for the canonical WNT signaling pathway, is significantly up-regulated in all brain regions we tested from mice exposed to territorial threat. Human TCF7L2 has been linked to metabolic disorders and neuropsychiatric disease through GWAS studies and the dual role of this TF in both metabolic and behavioral response has been confirmed in transgenic mice. However, the regulatory targets and functions of Tcf7l2 have not been identified in brain, and the regulatory activities of this WNT effector have never been analyzed in the context of behavior. We have identified Tcf7l2's regulatory targets and adult brain expression patterns and compared its activities in mouse brain regions in response to social threat. These data provide new answers to the role of WNT signaling in social learning and provide new clues to the link between metabolism and behavior. These findings may also lead to increased understanding of and new treatments for social disorders already linked to Tcf7l2, such as Autism and Schizophrenia.

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52. Development of a Mouse Model of Hyper Caloric Diet and Ethanol intake to Study Addiction Overlapping

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The seeking behaviour for hyper-caloric foods and drugs of abuse, such as ethanol (EtOH), are governed by the reward system (RS) in the brain. We have developed an animal model fed with a hyper-caloric diet and EtOH in an effort to understand how changes in the standard transcriptional regulation of genes from the mesolimbic dopaminergic pathway are involved in the development of compulsion for high-calorie foods and EtOH. In the first experimental stage (T1), the animals were fed for 4 weeks with a standard diet (AIN93G) or a high sugar and butter (HSB) diet. At the start of T2, the free-choice protocol between water and 10% EtOH solution was introduced, along with the dietary treatment. During T2, HSB diet was replaced by the AIN93G diet in some groups. We show that HSB diet induced lipogenesis, weight gain and increased serum leptin concentrations. In addition, consumption of HSB diet changed EtOH preference patterns. The increase of this preference may have been triggered by the

changes in transcriptional regulation patterns of genes involved in the RS: up-regulation of the Gabbr1 subunit and down-regulation of the Gabbr2 subunit of the GABAB receptor; up-regulation of Rdx (Radaxin) and down-regulation of the Cltc (Clathrin Heavy Chain). Thus, it is plausible that the animal model described has compulsion for high-calorie diet and an increased preference for EtOH when the hyper-caloric diet is withdrawn. The model allowed the study of the RS and differential regulation of its genes in the context of binge eating and EtOH consumption.

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54. Examining 25 classic schizophrenia candidate genes in the context of GWAS data – evidence for relevance?

Emma Johnson [1,2], Whitney Melroy [3], Richard Border [1,2], Matthew Keller [1,2*], Marissa Ehringer [1,4*]

Farrell et al (2015) recently investigated how well evidence for each of the 25 most-studied candidate gene polymorphisms in schizophrenia literature replicate in the Psychiatric Genomic Consortium's (PGC) genome-wide SNP GWAS. In the current project, we expand their findings by examining whether there is evidence that these 25 previously studied candidate genes themselves, as opposed to the specific candidate polymorphisms, have stronger associations with schizophrenia than would be expected by chance. To test this, we are using the collection of minimum SNP p-values within each of the 25 schizophrenia candidate genes from the PGC GWAS on schizophrenia, and comparing this distribution to the distribution of p-values derived from various sets of 25 control genes. These sets of control genes were matched to our 25 test genes on several parameters: gene length, SNP density, expression in the brain, and hits in other (non-psychiatric trait) GWAS.
Our principle aim is to address the following question: when we compare the results of schizophrenia candidate gene research to hypothesis-free GWAS findings, is there evidence that investigators focused on biologically-relevant, confirmed by GWAS, candidate genes for schizophrenia?
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Support: NIH grants R01 AA017889, R01 MH100141, T32 HD007289, T32 MH06880

56. Identifying mouse models for neurodevelopmental defects in attention deficit-hyperactive disorder

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Mouse Genome Informatics (MGI, http://www.informatics.jax.org) provides free access to mouse genetic, genomic and biological data in support of mouse as a model for human biology and disease. There are over 710,000 mutant alleles in almost 22,000 genes represented in MGI with 47,000 of these mutant alleles representing over 11,000 unique genes in live mice (the remainder existing in cell lines only). Many of these mutations are associated with human disease (over 4,800 mouse genotypes modeling over 1,400 OMIM diseases) and their reported phenotypes are carefully curated with the Mammalian Phenotype ontology (>291,000 annotations to 56,153 genotypes). These contribute to and are integrated with the vast body of other biological data in MGI. Using MGI search tools, we are able to extract information on known disease causing genes, mouse models, existing literature associated with the published data, and identify new potential human disease models.
Attention deficit-hyperactive disorder (ADHD) is a neurodevelopmental disorder characterized by difficulty in paying attention, impulsive actions, hyperactivity, and often accompanied by other behavioral and psychological conditions. Although there is no consensus on the prevalence of ADHD, reported cases per year have been consistently on the rise and currently cost Americans close to $50 billion per year. As the most commonly diagnosed neurological disorder in childhood, there is much active research on ADHD with close to 30,000 research articles listed in PubMed. In a continuing effort to map the causative gene(s) for ADHD to better understand the disorder and potential target for gene therapies, researchers have identified mutations in two human genes, *DRD4* and *DRD5*, to be involved in the disorder and an additional four susceptibility loci. Here, we will present the utility of MGI tools to search for mouse mutations that model this human disease. By comparing mouse gene and disease model phenotype data in MGI, we have identified mutations in 9 mouse genes and 13 models for ADHD. All of these genes have not yet been associated with their human ortholog as causative genes in human ADHD.

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Support: MGI is supported by NIH grant HG000330.

58. Piezo system identifies genes influencing sleep from KOMP2 pipeline with a high hit rate

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Our current study employs a non-invasive, high throughput piezoelectric system that characterizes sleep-wake phenotypes in a large population of control and single-gene knockout mice; recorded as part of the Knockout Mouse Phenotype

Program (KOMP2) at JAX. A piezoelectric sensor pad placed at the bottom of the mouse cage records gross body movements. The pressure signals thus generated are classified by an automated classifier into sleep and wake. The system characterizes sleep traits over 24 hours, as well as during the light and dark phase. The system has been well validated over many years, and matches approximately 95% with EEG and 90-95% with human observation. Quality control is ensured by a data confidence metric, automating the rejection of any poor quality data. Newer algorithms also allow sleep to be differentiated into REM vs. non-REM sleep. To date, over 6500 mice representing nearly 300 knockout lines, and more than 1000 control mice have been recorded in addition to many inbred mouse strains for more than 200 physiological and behavioral phenotypes that allow both known and unknown correlations to be assessed across the KOMP2 population. Thus far, 38 candidate sleep related genes have been identified that produce significantly altered sleep phenotypes, depending on the specific sleep traits assessed. Some genes were found to specifically alter total sleep amounts or sleep fragmentation (sleep bouts) specifically during the light phase or dark phase. The piezo system utilizes the breathing pattern to assess sleep vs. wake, and several genes were also found that alter breathing variables.

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60. Brain-specific Allelic Imbalance of *Trappc9* Influences Metabolism and Behavior

Liang ZS[1], Raghupathy N[2], Munger SC[2], Logan DW[1]

Parent-of-origin allelic bias, the epigenetically regulated preferential expression of the paternally- or maternally-derived allele of a gene, is prevalent in the mammalian brain but little is known about its influence on physiology and behavior.

We hypothesize that the olfactory system is an unexplored and promising target for parental allelic bias in the nervous system. We examined the transcriptomes of the olfactory bulb (OB) and the main olfactory epithelium (MOE), using reciprocal crosses of two distantly related inbred mouse strains, CAST/EiJ and C57BL/6J, by RNA sequencing. We quantified the allele-specific expression of over 10,000 genes, resulting in the identification of over 50 candidate genes with reproducible and reciprocal parent-of-origin biases. One such candidate, Trappc9, encodes a mammalian ER-Golgi trafficking protein, biallelically expressed except in the brain, where ~70% of transcripts are generated from the maternal allele. We studied a mouse mice line specifically lacking the maternal, paternal or both alleles of *Trappc9* to determine the phenotypic effect of its parent-of-origin allelic bias. We found *Trappc9* $^{m-/p-}$ mice display pathological features of obesity with microcephaly, and a reduction in exploratory activity, Mice lacking a maternal allele (*Trappc9* $^{m-/p+}$), displayed a similar pathology on

metabolism and behavior. Those lacking a paternal allele (*Trappc9* $^{m+/p-}$) display none of these phenotypes. Individuals with homozygous truncating mutations in human TRAPPC9 result in obesity and a rare intellectual disability disorder. Together, these analyses demonstrate that parent-of-origin allelic bias in the brain can have significant functional consequences in the control of metabolism and cognition. Further work will be necessary to determine whether the knockout mice have cognitive deficits, whether human TRAPPC9 also has an allelic imbalance in the brain and whether it too generates a parent-of-origin derived phenotype.

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62. The role of JmjC demethylases in alcohol-related behaviors

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Alcohol exposure affects gene expression in the brain and can lead to addiction in humans and other model organisms. Epigenetic changes act on DNA or Histones to alter the transcription, or expression, of genes. Histone modifications include both acetylation and methylation; the latter was considered stable until the discovery of histone demethylases (KDM). Among KDMs, a group of enzymes with a jumonji C (JmjC) domain appear to fine-tune developmental processes, but its role in alcohol exposure is not known. Histone methylation/demethylation can promote or silence transcription, maintaining the balance of cellular processes or generating the appropriate response to stimuli. Given that alcohol abuse is a cause of health deterioration, it is therefore essential to understand the effects of demethylation in response to alcohol exposure. In this study, we explore the behavioral changes related to alterations in JmjC demethylase genes in *Drosophila melanogaster*. Sedation and tolerance towards alcohol were measured on flies with mutations on JmjC demethylase genes. Our results indicate that lack of NO66, KDM3, and lid, decrease the resistance to sedation in the flies. Lack of lid and NO66 results in increased tolerance contrary to mutations in KDM3 that resulted in reduced tolerance. These three demethylases interact with *snr1*, (chromatin modification mediator in humans and worms) which has interactions with various genes involved in alcohol-related behaviors. These results suggest JmjC demethylases play a significant role in alcohol consumption and further studies are needed to determine additional effects and their role in alcohol addiction.

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64. Methodological research : development of primers specific to X & Y gametolog genes in the context of psychiatric disorders sexual dimorphism

Pascalina Suciu

The frequency of occurrence of psychiatric and neurodegenerative disorders is known to vary between sexes in human. Furthermore, sexual differences have been indicated to be manifested already during embryonic development through genetic determinants. Gametolog genes encoded on the X and Y chromosome have been highlighted to be good candidates for underlying this dimorphism, including ZFX, ZFY, PCDH11X, PCDH11Y, PRKX, PRKY, USP9X, USP9Y, DDX3X, DDX3Y, UTX, UTY, TMSB4X, TMSB4Y, NLGNX, NLGNY, TXLNGX, TXLNG2P, KDM5CX, KDM5CY, EIF1AX and EIF1AY. To investigate expression differences for these genes between female and male embryos we have developed a multi-mismatches nested strategy that allows the design and test of highly specific primers for the X or Y gametologs. This methodology provides advantages over RT-qPCR as the high sequence conservation between the X and Y gene pairs makes it impossible to design specific primers for these genes with ordinary primer settings to obtain 100% confidence in the X and Y specificity of the primers. In this work we are describing a new research tool including 11 sets of highly specific primers ready for use in both Homo sapiens and Pan troglodyte for the investigation of sexual dimorphism in psychiatric neurodegenerative disorders. Investigating the genetic basis of these dimorphisms could offer new insights in the underlying mechanisms of the development of the studied disorders. Moreover, it could provide information about the roles of the gametolog genes themselves. Moreover, these primers can also be used for assessments in post-embryonic stages, as it is the case currently, in the context of Alzheimer and Schizophrenia studies.

Uppsala University, Uppsala, Sweden

Sunday May 15, 2016

Symposium 4: RNA binding proteins in neural development, plasticity and psychiatric disorders

Sam68 in neuronal function and brain disorders

Matthew E. Klein[1], Thomas J. Younts[1], Guoan Zhang[2], Thomas A. Neubert[2], Pablo E. Castillo1, Bryen A. Jordan[1]

Learning and memory require activity-dependent regulation of gene expression. However the mechanisms that localize the products of gene expression to specific synapses within complex neuronal morphologies are poorly understood. The targeted transport and translation of mRNAs by RNA binding proteins (RBPs) represents an attractive mechanism to explain input-specific regulation of synaptic function. Aberrant RBP function has been linked to numerous

neurodegenerative and developmental disorders including autism and Fragile-X-syndrome (FXS). We found that neuronal activity increases the synaptic localization of diverse RBPs including Sam68 (Src associated protein in mitosis, 68kD), which has been associated with fragile X tremor/ataxia syndrome (FXTAS). Sam68 regulates the localization of mRNA cargos in neuronal dendrites and promotes their translation through enhanced association with synaptic polysomes, which differs from well-known RBPs that *repress* translational such as FMRP. Our data suggests that Sam68 and FMRP share mRNA cargos, suggesting that they oppositely regulate a common pool of mRNAs. We recently found that Sam68 is required for mGluR-dependent translation of Arc (activity-regulated cytoskeleton associated protein/Arg3.1) and mGluR-dependent long-term depression (mGluR-LTD), but only in distal dendrites of hippocampal CA1 regions, suggesting that Sam68 regulates local function. Our experiments provide insights into the mechanisms that regulate the temporal and spatial expression of proteins within neurons and their role in input specificity. Moreover, finding that Sam68 and FMRP oppositely regulate a common pool of mRNAs would have far-reaching implications on our understanding of FXS, as loss of FMRP may lead to unbalanced Sam68 function.

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The retrotransposon storm: Retrotransposon activation causes neurodegeneration in a *Drosophila* TDP-43 model of amyotrophic lateral sclerosis.

Krug, L.[1,2], Chatterjee, N.[1], Borges-Monroy, R.[1,3], Hearn, S.[1],Theodorou, D.[1,4], Dubnau, J.[1,2]

Functional abnormality of TDP-43, an aggregation-prone RNA binding protein, is observed in the vast majority of both familial and sporadic cases of amyotrophic lateral sclerosis (ALS), but the mechanism by which cell death occurs is not understood. We advance the novel hypothesis that loss of control of Retrotransposons (RTEs) contributes to TDP-43 pathology. This hypothesis is founded on a series of observations: We previously demonstrated a deterioration of RTE suppression – and resultant RTE activity – with advancing age in the *Drosophila* brain. Second, we recently demonstrated via meta-analysis of deep sequencing data that TDP-43 protein binds promiscuously to RTE–derived transcripts in rodent and human brain, and that this binding is lost in cortical tissue of patients. We now demonstrate in a *Drosophila* model that TDP-43 mediated degeneration is caused by disruption of RTE silencing, resulting in a storm of activated RTEs that leads to DNA damage and apoptotic cell death. We show that hTDP-43 toxicity impairs small interfering RNA (siRNA) silencing, which is the major post-transcriptional mechanism of RTE control in somatic tissue. This causes early and severe activation a specific RTE, the endogenous

retrovirus (ERV) *gypsy*. We further demonstrate that *gypsy* causes degeneration because we are able to rescue hTDP-43 toxicity by concomitantly blocking expression of this RTE. Moreover, we provide evidence that activation of DNA damage-mediated apoptosis underlies hTDP-43 toxicity, consistent with RTE-mediated effects. Our findings suggest that RTEs drive neurodegeneration in TDP-43-mediated diseases such as ALS.

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Transcriptional and splicing networks associated with methamphetamine behavioral and neuroanatomical dysfunction in *Hnrnph1* (heterogeneous nuclear ribonucleoprotein H1) knockouts

Camron D. Bryant

We identified *Hnrnph1* as a quantitative trait gene for the stimulant and rewarding properties of methamphetamine (Yazdani et al., 2015; *PLOS Genetics*; see 2016 poster), joining the list of several RNA binding proteins (RBPs) that have recently been implicated in addiction (Bryant and Yazdani, 2016, *G2B*). hnRNP H1 is a nuclear RBP involved in post-transcriptional regulation; thus, identifying the brain splicing targets of hnRNP H1 could inform molecular mechanisms underlying neurobehavioral function in response to psychostimulants. Here, we conducted RNA-seq analysis of the striatum from *Hnrnph1* $^{+/-}$ mice versus wild-types to identify the transcriptome and spliceome associated with neurobehavioral dysfunction. Ingenuity Pathway Analysis identified neurological disease/movement disorders as the top category for both the transcriptome (224 genes, $p=5.72x10^{-50}$) and spliceome (190 genes, $p=4.3x10^{-42}$) - 74 of these genes overlapped. Interestingly, the top upregulated gene was *Cnr1* (cannabinoid receptor 1), showing a 1.3-fold upregulation in *Hnrnph1* $^{+/-}$ mice ($FDR=3.3x10^{-13}$). To identify the direct RNA targets of hnRNP H1, we are generating floxed *Hnrnph1* knockin mice containing an HA tag for CLIP-seq studies. Furthermore, to test the hypothesis that *Hnrnph1* $^{+/-}$ mice show a perturbation in the dopamine circuitry, we are examining tyrosine hydroxylase staining in midbrain dopamine neurons and their forebrain projections. Finally, we are examining activity-dependent cellular localization of hnRNP H in cultured neurons following neuronal stimulation with KCl, D1, and D2 dopamine receptor agonists. These studies are providing new insight into the neural function of hnRNP H1 and will further our understanding of neuronal disorders involving dopamine neurotransmission.

Director, Laboratory of Addiction Genetics, Department of Pharmacology and Experimental Therapeutics and Psychiatry, Boston University School of Medicine

Control of drug-related plasticity by the fragile X mental retardation and activity-regulated cytoskeleton-associated proteins

LN Smith[1], JP Jedynak[1], RD Penrod[1], J Kumar[1,4], MM Thomsen[1], MR Fontenot[4], CF Hale[3], KC Dietz[2], FS Thomas[2], M Taniguchi[1], BC Zirlin[1], KM Huber[3], SG Birnbaum[2], MJ Thomas[5], CW Cowan[1]

Chronic exposure to drugs of abuse establishes persistent, maladaptive changes in behavior, as well as in synaptic structure/function. Here we show that the fragile X mental retardation protein (FMRP), an RNA binding protein, and one of its targets, the activity-regulated cytoskeleton-associated protein (Arc), play critical roles in regulating behavioral and synaptic plasticity resulting from psychostimulant exposure. While *Fmr1* knockout (KO) mice show significant reductions in drug-related behaviors compared to wild-type littermates, *Arc* KO mice show responses consistent with increased sensitivity. We establish a post-developmental role for FMRP in drug-induced adaptation in the nucleus accumbens (NAc), and in accordance with FMRP's role in synapse weakening/elimination, find that cocaine enhances structural and functional connectivity in NAc shell medium spiny neurons of Fragile X mice. Our findings help explicate FMRP and Arc's roles in experience-dependent plasticity and offer insight into mechanisms underlying pertinent neuropsychiatric disorders, such as drug addiction and Fragile X.

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Support: NIDA T32 DA007290, F32 DA027265, DA008277, DA027664, DA030590, DA019666, R21 DA033457, and K02 DA035459, NINDS NS062158, FRAXA Research Foundation, and Simons Foundation Autism Research

Selected Talks 3: Featured Speaker

Commonalities across diverse species reveals deeply conserved mechanisms underlying social response

Lisa Stubbs[1,2], Michael Saul1, Christopher Seward[1,2], Hagai Shpigler[1,3], Abbas Bukhari[1,4], Laura Sloofman[1,5], Joseph Troy[1,6], Huimin Zhang[1,2], Amy Cash Ahmed[1,3], Xiaochen Lu[1,2], Jian Ma[1,7], Sihai Dave Zhao[1,8], Alison Bell[1,4], Saurabh Sinha[1,5], Gene Robinson[1,3]

Animals display a wide diversity of social behaviors, adapted independently to distinct environments, reproductive strategies, and many other types of species-specific biological traits. Despite this diversity, certain types of social responses

are widely shared. Several lines of evidence have suggested that those common behaviors share common molecular mechanisms operating in functionally similar neurons and supporting cell types, even in animals as divergent in morphology and evolutionary history as insects and mammals. To explore the extent of these fundamental commonalities, our collaborative team has examined gene expression and epigenetic patterns in the brains of three diverse behavioral model species: honey bee, stickleback fish, and laboratory mice. We have examined two distinct behavioral paradigms, involving territorial threat on the one hand, and offspring nurturance on the other, and analyzed genome-wide expression and epigenetic profiles at various time points after exposure across interconnected, socially responsive regions of the brain. Analysis of these data has required the development of new resources, experimental methods and analytical approaches to facilitate the detection of related processes across such a deep species divide. Application of these methods to the rich datasets we have created reveal the existence of ancient, deeply conserved genetic networks that control a remarkably similar series of events to trigger emotional learning and adaptive behavioral response.

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Selected Talks 3: Speaker 2

Transcriptional regulatory dynamics underlay metabolic and neural responses to a social threat in mice

*Michael Saul[1], *Christopher Seward[1,2], Joseph Troy[3], Laura Sloofman[4], Patricia A. Weisner[5], Derek Caetano-Anolles[2], Huimin Zhang[1], Hao Sun[1], Yang Zhang[6], Dave Zhao[1,7], Jian Ma[6], Sriram Chandrasekaran[8], Saurabh Sinha[1,4,9,10], Lisa Stubbs[1,2,5]

Agonistic encounters with conspecifics are powerful effectors of future behavior. The classic resident-intruder paradigm wherein a territory holder experiences a social challenge from an intruder evokes strong, durable responses in neurobiology. Our group recently identified a “toolkit” of transcriptional regulatory elements underlying the response to social challenge conserved across phyla. The present work expands these results, tracking the neural genomic response to challenge in mice across regions and time points after social challenge. We assayed frontal cortex, hypothalamus, and amygdala, at 30, 60, and 120 minutes after social challenge. Using RNA-Seq, we compared challenged and control animals in each tissue-time combination. We identified complex spatiotemporal patterning of differential expression enriched for genes classically associated with insulin, neural, and developmental signaling. Cell type deconvolution correlated these genes with neurons. A second analysis examining the interplay between brain regions and times after challenge found challenge-associated

differential expression enriched in oxidative phosphorylation. Cell type deconvolution correlated these genes with oligodendrocytes. Using transcriptional regulatory analysis, we found that a network of transcription factors including *Esrra*, *Lhx2*, *Neurod2*, and *Pparg* predicted challenge-associated differential expression. Using ChIP-Seq on H3k27Ac marks, we found that the combination of open chromatin and the transcription factor binding sites of some of these transcription factors strongly predicted differential expression. Further, ChIP on *Esrra* identified many peaks related to the oxidative phosphorylation signature. This dataset shows the rich neural responses to social challenge and validates its underlying regulatory drivers with unprecedented tissue and temporal resolution.

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Support: Simons Foundation #SFLife 291812, New York, NY, USA.

Selected Talks 3: Speaker 3

High Heritability of Social Visual Engagement in an Epidemiologic Twin Sample: Implications for Autism and Typical Development

John N. Constantino[1], Stefanie Kennon-McGill[1], Claire Weichselbaum[1,2], Natasha Marrus[1], Alyzeh Haider[1], Anne L. Glowinski[1], Ami Klin[3,4,5], Warren Jones[3,4,5]

Individual variation in how children perceive their social environment may have important implications for social development and functioning. Reduced eye contact and lack of attention to social cues are classic symptoms of autism spectrum disorders (ASD). Recent studies have quantified these phenotypes using eye-tracking technology during viewing of dynamic social scenes, and found that such measures of social visual engagement (SVE) can predict later autism diagnosis in children with familial ASD risk, raising the possibility of an autism endophenotype. However, the heritability of this measure remains unexplored. Here we describe the first study of SVE in an epidemiologic sample of 18- to 24-month-old twins (n=168) and report a remarkable degree of monozygotic twin-twin concordance (0.90), with lower dizygotic concordance (0.30). These findings reveal a surprising amount of genetic influence on the social engagement of young children, informing our understanding of both typical and autistic social development.

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Selected Talks 3: Speaker 4

Characterizing glucocorticoid receptor signaling with mutant zebrafish strains in the HPA axis

Han B. Lee[1], Tanya L. Poshusta[2], Bethany C. Berry[2], Randall G. Krug II[1], Makayla R. Berg[2], Ashley N. Sigafoos[2], Brynn N. Sundberg[2], Cassandra E. Bullard[2], and Karl J. Clark, Ph.D.[1,2]

A hallmark pathophysiological change in neuropsychiatric disorders is alterations in the hypothalamic-pituitary-adrenal (HPA) axis activity. The HPA axis mediates vertebrate-specific systemic stress response (SR) through biphasic glucocorticoid receptor (GR) signaling comprising rapid non-genomic and slower genomic responses. There is knowledge gap on how changes in non-genomic GR response lead to lasting alterations in genomic response. We hypothesized that zebrafish locomotor response to hyperosmotic stimulation (100 mM NaCl) is dependent on the HPA axis and rapid non-genomic GR signaling. Significantly, this larval zebrafish locomotor assay provides a versatile platform that can dissect molecular components of non-genomic glucocorticoid signaling independently from any genomic response. By measuring locomotor response, genomic GR activation, cortisol levels, and drug response to selected antagonists using zebrafish mutants in the HPA axis, we have demonstrated that the locomotor response is dependent on *mc2r* (adrenocorticotropic hormone receptor). Both GR somatic mutants injected with TALENs and GR germline mutants have significantly decreased genomic response shown by the level of GR transcripts and glucocorticoid response element activation. However, rapid locomotor response to hyperosmotic stress is not decreased in GR germline mutants whereas significantly decreased in GR-targeting TALEN-injected fish. We are investigating translational GR isoforms potentially responsible for non-genomic response in germline mutants by targeting alternative loci in GR with different TALENs and TALEN-mediated precision knock-in strategies. Dissecting non-genomic and genomic GR response with genetic perturbation may lead to an establishment of a versatile assay system to investigate vertebrate SR and identification of components in non-genomic GR response.

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Selected Talks 3: Speaker 5

A spontaneous mutation of *Neurexin3* in the 129S1/SvImJ strain of mice enhances empathic fear behavior.
Sehoon Keum, Arie Kim, and Hee-Sup Shin

Empathy is an important emotional process that involves the ability to recognize and share emotions with others. We previously developed an observational fear learning (OFL) behavioral assay to measure empathic fear in mice. In the OFL task, a mouse is conditioned for fear to the context where it observes a conspecific demonstrator receiving aversive stimuli. We have recently reported that empathic fear response is highly variable among 11 inbred mouse strains, and innate differences in conditioned fear, anxiety, locomotor activity, sociability and preference for social novelty are not significantly correlated with OFL among those strains. However, the genetic factors underlying variability in empathic fear remain to be determined. Intriguingly, we have found that mice of the 129S1/SvImJ (129S1) strain, exhibit a marked increase in OFL, as compared with another 129S substrain, 129S4/SvJaeJ (129S4). Through genetic and molecular analyses, a nonsynonymous mutation of arginine to tryptophan (R498W) in *Neurexin 3* (*Nrxn3*) was identified as the causative variant. This mutation occurs at a residue that is well conserved among mammalian species and is predicted to be deleterious to the protein by *in silico* databases. We have further determined that a deletion of *Nrxn3* in the anterior cingulate cortex leads to a decrease in OFL. Knock-in mice with the R498W mutation produced by the CRISPR/Cas9 system is currently being tested. Taken together, we propose that *Nrxn3* is an important regulator in neural circuits of OFL. These works also demonstrate the validity of the approach to utilize substrains to identify genes and alleles regulating social behaviors.

Center for Cognition and Sociality, Institute for Basic Science (IBS), Daejeon, Republic of Korea

Monday May 16, 2016

Distinguished Scientist: Guoping Feng

Dissecting Synaptic and Circuitry Mechanisms of Autism
Guoping Feng,

Recent genetic studies have identified a large number of candidate genes for autism spectrum disorder (ASD), many of which encode synaptic proteins, suggesting that synaptic dysfunction might be a key pathology in ASD. In addition, recently, genetic studies have revealed a significant overlap of risk genes for ASD and schizophrenia. However, it is not clear how different mutations of the same gene could contribute to the manifestation of different diseases. One such example is the Shank3 gene. The Shank3 gene encodes a postsynaptic scaffolding protein critical for the development and function of glutamatergic excitatory synapses. Disruption of the Shank3 gene is thought to

be the cause of the core neurodevelopmental and neurobehavioral deficits in Phelan-McDermid Syndrome, an autism spectrum disorder. Using various Shank3 mutant mice as a model system, I will discuss (1) recent findings on synaptic and circuit mechanisms underlying autistic-like behaviors in Shank3 mutant mice; (2) the reversibility of synaptic, circuit and behavioral abnormalities in adult mutant mice; and (3) molecular and synaptic mechanisms that may explain how different alleles of the same gene lead to distinct synaptic and behavioral phenotypes in mice. Together, these findings may inform exploration of neurobiological mechanisms of ASD in human patients.

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Featured Speaker – Elinor Karlsson

Shared genetics of obsessive compulsive disorder in dogs and humans

EK Karlsson[1,2], HJ Noh[2], J McClure[1,2], D Genereux[1,2], G Feng[2,3], K Lindblad-Toh[2,4]

Canine OCD is a naturally occurring model for human obsessive compulsive disorder. In both species, OCD manifests as time-consuming repetition of behaviors causing functional impairment, with similar age of onset and response to treatment with SSRIs. In the first GWAS of canine OCD, we found significant association to the neural cadherin CDH2. By deep sequencing the top GWAS regions in diverse breeds, we found three more genes acting in glutamatergic signaling pathways and identified two candidate variants between CDH2 and DSC3 that disrupt gene regulation in a human neuroblastoma cell line. We combined data from dog and mouse studies to design a human association study (592 cases and 560 controls) targeting 608 genes in OCD associated pathways. We leveraged evolutionary and functional annotations to identify rare, potentially causative functional variants, including seven missense variants in postsynaptic protein-binding domains of NRXN1, and 17 regulatory variants in CTTNBP2 and REEP3, six of which alter transcription factor binding.
We show that genetic mapping in dogs, one of the best natural models for behavior, can find new genes and pathways implicated in OCD. Furthermore, we illustrate how animal models and functional annotation used in concert can identify potentially causative functional variants even with limited genomic data. We have now launched a citizen-science driven dog behavioral genetics project, dubbed “Darwin’s Dogs”. By enrolling any dog, regardless of ancestry, and collecting rich phenotype data from owners, “Darwin’s Dogs” will allow well powered studies of complex behavioral traits in dogs to be done quickly and efficiently.

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Featured Speaker – Kyung-An Han

Gene-social context-environment interaction critical for response inhibition

Paul Sabandal, Erick Saldes, Youngcho Kim, John M. Sabandal, and Kyung-An Han

The ability to suppress ongoing motor actions that are no longer appropriate is a fundamental feature of executive function supporting flexible and goal-oriented behaviors. This inhibitory control known as response inhibition has been studied in mammals but not in non-mammalian species. We have identified that the fruit fly *Drosophila* displays response inhibition. A go/no-go test is typically used to measure response inhibition in human subjects and requires subjects to produce a motor response when cued to do so (go) or otherwise withhold it (no-go). To study response inhibition in *Drosophila*, we have developed a fly version of the go/no-go test and found dopamine as an important neuromodulator for inhibitory control. When subjected to a go/no-go test, the *fmn* mutants lacking dopamine transporter initially withheld movement but, within a minute, exhibited loss of inhibitory control. The phenotype is sensitive to the social context, offering a useful model to study the mechanism by which genetic, social and environmental factors impinge on response inhibition. Anomalous response inhibition is associated with numerous brain disorders including addiction, ADHD, autism and PTSD but its underlying mechanism is largely unknown, and our study may help fill the knowledge gap.

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Selected Talks 4: Speaker 1

Quantitative Trait Locus Mapping of Oxycodone Reward and Naloxone Aversion in C57BL/6 Substrains

Lisa R. Goldberg[1], Stacey L. Kirkpatrick[1], Neema Yazdani[1], Megan K. Mulligan[2], and Camron D. Bryant[1]

Opioid addiction is heritable, yet its genetic basis remains poorly understood. Mice are valuable for identifying novel genes that contribute to variation in traits associated with various stages of addiction, including acute opioid-induced psychomotor stimulation and conditioned reward. The closely related C57BL/6J and C57BL/6NJ strains exhibit limited genetic diversity, yet show significant

strain differences in several traits, including ethanol consumption, psychostimulant behaviors, and naloxone conditioned place aversion. Quantitative Trait Locus (QTL) mapping in these substrains drastically reduces the number of segregating genetic variants from millions to thousands, accelerating the identification of the causal genetic factors. We conducted QTL mapping for oxycodone conditioned place preference (OXY, N=198), and naloxone conditioned place aversion (NAL, N=205), along with saline-treated mice as controls (SAL, N=193). We utilized a 9 day CPP/CPA protocol. Mice received drug (1.25 mg/kg OXY, 4 mg/kg NAL, or SAL) on D2 and D4, and SAL on D3 and D5. Mice were assessed for drug-free CPP/CPA (D8) and drug state-dependent CPP/CPA (D9). QTL mapping was performed in R/qtl (scanone, 1000 permutations) using 96 informative markers. Preliminary analyses identified two overlapping OXY-specific QTLs for D2 (Ch1 72.43 cM, $p<0.05$) and D4 locomotor (Ch1 72.43 cM, $p<0.05$). Additionally, we identified two overlapping NAL-specific QTLs for state-dependent CPA (Chr 18 29.22 cM, $p<0.1$) and D9 freezing bouts on the NAL-paired side (Chr 18 29.22 cM, $p<0.05$). We are currently identifying cis-eQTLs from striatal tissue using mRNA sequencing to further aid in candidate gene identification and validation via gene editing.

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Support: R00DA029635 (NIDA; C.D.B.), R03DA03828702 (NIDA; C.D.B), Transformative Training Program in Addiction Science (Burroughs Wellcome 9550300872)

Selected Talks 4: Speaker 2

Genome-wide mapping in the Diversity Outbred Population

Kayvon Sharif[1], Troy Wilcox[2], Dan Gatti[2], Eric Busch[3], Emily Funsten[1], Steven Kasparek[1], Drew Kreuzman[1], Benjamin Mansky[1], Sophie Masneuf[3], Erica Sagalyn[3], Dominik Taterra[1], Walter Taylor[1], Mary Thomas[1], Andrew Holmes[3], Elissa J. Chesler[2], Clarissa C. Parker[1]

A strong predictor for the development of alcohol use disorders (AUDs) is altered sensitivity to the intoxicating effects of alcohol. Individual differences in the initial sensitivity to alcohol are controlled at least in part by genetic factors, yet finding the specific genes that underlie these differences has proven difficult. Mice offer a powerful tool for elucidating the genetic basis of behavioral and physiological traits relevant to AUDs; yet conventional experimental crosses have only been able to identify large chromosomal regions rather than specific genes. Genetically diverse, highly recombinant mouse populations allow for the opportunity to observe a wider range of phenotypic variation, offer greater mapping precision, and thus increase the potential for efficient gene identification.

We used newly developed Diversity Outbred (DO) mice to fine-map quantitative trait loci (QTLs) associated with ethanol sensitivity. We phenotyped 778 DO mice for ethanol-induced hypothermia. We used the GIGAMUGA to genotype a subset (N = 288) of these mice at ~140k SNP markers across the genome and performed high precision QTL mapping using DOQTL. A repeated-measures ANOVA indicated that following ethanol administration, subjects showed significant changes in body temperature over time, $F(3.0, 1968.8) = 1098.3$; $p < 0.0001$, $= 0.59$. Our preliminary QTL analyses identified several suggestive and significant QTLs associated with ethanol-induced hypothermia, thus supporting the utility of DO population as a powerful tool for fine-mapping. The novel genetic mechanisms identified using the DO may hold translational potential in identifying biological mechanisms for AUDs.

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Selected Talks 4: Speaker 3

Novel animal models of initial cocaine sensitivity using Collaborative Cross mice

S Adams Schoenrock[1,2], J Farrington[1], FP Manuel de Villena[3,4,5], W Valdar[3,5] , LM Tarantino[1,6]

Substance use disorders (SUDs) are highly prevalent and impose a substantial burden on society. Although these disorders are highly heritable, identifying specific genes and mechanisms in humans has been difficult due to symptomatic heterogeneity and inability to perform baseline assessments in humans after prolonged drug exposure. Animal models of specific aspects of SUDs allow for assessment of underlying mechanisms and genetics. Novelty-induced locomotion has been used to predict initial sensitivity and self-administration of psychostimulants. However, the link between these behaviors has varied across studies and identification of underlying mechanisms has been challenging. We identified two Collaborative Cross Recombinant Inbred Intercross (CC-RIX) lines that showed significantly high (CC-RIXhigh) and low (CC-RIXlow) locomotor response to novelty. We predicted and confirmed that initial locomotor response to cocaine is also higher in CC-RIXhigh animals compared to CC-RIXlow. We believe these two strains can be utilized as models of high and low predisposition for initial drug sensitivity. We have designed a set of experiments to further characterize these strains for a range of addiction-related behaviors and begin to elucidate the underlying mechanisms for these phenotypic differences. These studies include investigation of stress response, dopaminergic pathways and

pharmacokinetics. Additionally, we are using the unique genetic makeup of CC lines to map candidate regions underlying divergent cocaine responses. We believe that utilizing Collaborative Cross mice will enhance our ability to examine the link between initial drug use and progression to addictive-like behaviors due to the genetic diversity and availability of resources that enable systems genetic approaches in these strains.

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Suuport: R01MH100241 from the National Institute of Mental Health, National Institutes of Health

Selected Talks 4: Speaker 4

Neural Dynamics of the Decision to Drink in Alcohol Preferring 'P' and Wistar Rats

David N. Linsenbardt, & Christopher C. Lapish

Neural activity within the prefrontal cortex (PFC) is robustly altered by presentation of environmental stimuli associated with alcohol, and is correlated with alcohol craving, relapse, and mediated by genetic risk. However, we know very little about how genetic risk for excessive drinking influences the processing of alcohol-related cues across ensembles of neurons within the PFC. Alcohol-preferring (P) rats and heterogeneous Wistar rats were used in a Pavlovian fluid self-administration task together with in vivo electrophysiology to determine the neural dynamics of cue-associated alcohol drinking. Ensembles of PFC neurons produced coherent stimuli-specific networks states capable of dissociating rat populations (P vs. Wistar) and the type of fluid made available (alcohol vs water). Furthermore, neural trajectories associated with stimuli-preceding fluid availability were used to identify subsets of neurons whose firing rate discriminated drinking from non-drinking trials (i.e. predicted drinking before it occurred). These data support increasing evidence that populations with a genetic vulnerability for excessive alcohol intake display altered processing of alcohol-associated cues, and highlight the PFC as a critical structure for the integration of alcohol-associated information and alcohol seeking/intake behavior.

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Support: This work was supported in part by grant #s: AA022268 (DNL), AA022821 (CCL), AA023786 (CCL), the ABMRF (CCL), and the Indiana Alcohol Research Center P60-AA007611 (D. Crabb).

Symposium 5: From Mouse Phenotype to Human Disease: integration and interpretation of behavior assays for disease relevancy.

Integrated analysis of KOMP2 behavioral data

Vivek Kumar, Donghyung Lee, Vivek Phillip, James Clark, Karen Svenson, Bob Braun, Stacey Rizzo, Elissa J. Chesler

Interpretation of model system data in the context of human disease has always been challenging. This is particularly difficult for behavioral assays that attempt to translate across complex disease states such as autism, schizophrenia, or addiction. The KOMP2 phenotyping pipeline at The Jackson Laboratory has characterized over 300 mouse knockout lines in 28 assays including 10 behavioral and 18 metabolic/physiology assays. This public dataset represents one of the largest using a classical rapid test battery approach. We have statistically modeled the data across all KOMP2 phenotypic domains and present an integrated analysis of KOMP2 data. We attempt to link the behavioral data with known disease phenotypes.

The Jackson Laboratory, Bar Harbor, ME 04609

Continuous recording of home-cage behaviours in group-housed mice; evaluation in selected strains and mutants

PM Nolan.

Our endeavour to record automated detailed behavioural parameters over time in an undisturbed cage environment encouraged us to explore whether true home-cage phenotyping was feasible, ie. could we monitor the behaviours of individual animals that are reared and group-housed in conventional IVC home-cages. To do this, we evaluated a system combining RFID tracking with infrared video recording for automated behavioural scoring. While RFID tracking allowed us to monitor spatial position, distance travelled and average pairwise distances between individuals, infrared video provided a means to extract the more complex behaviours of the animals at any point in the light-dark cycle. Moreover, we could track mouse behaviour in longitudinal studies spanning several days/weeks.

We first set out to explore behaviours in a number of mouse inbred strains to determine whether we could extract biologically meaningful differences using this system. Our findings show that individual strains can show multiple distinct behavioural patterns over 24-hrs in the home cage. This subset of home-cage behaviours has the potential to enrich existing behaviour phenotype ontologies. Secondly, we tested a number of relevant mutant lines to determine how discriminative these parameters were. Lines included models of neurodevelopmental, neurodegenerative, neuropsychiatric and metabolic disease as well as mutant lines showing deficits in activity, biological rhythms

and sleep. Our findings show that a far deeper understanding of mouse mutant phenotype can be established by monitoring behaviour in the home cage over one or more light-dark cycles.

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Informing Neurobehavioral Genetics: The International Mouse Phenotyping Consortium

Terrence Meehan[1] on behalf of the Mouse Phenotyping Informatics Infrastructure (MPI-2)

The International Mouse Phenotyping Consortium (IMPC) is building the first truly comprehensive functional catalog of a mammalian genome by producing and characterizing a knockout mouse strain for every protein-coding gene. Data from a standardized, broad-based phenotyping pipeline is collected and archived centrally by the IMPC-Data Coordinating Center. Dedicated ‘data wranglers’ are working with each phenotyping center to ensure proper transfer and quality control of data. A sophisticated statistical analysis pipeline identifies knockout strains with significant changes while accounting for bias from center and other confounding effects. Annotation with biomedical ontologies allows biologists and clinicians to easily find mouse strains with phenotypic traits relevant to their research and facilitates integration with other resources. With phenotype data now available for over 2500 genes, this talk will focus on the unique challenges of detecting abnormal neurobehavioral phenotypes, our plans for multi-parametric assessment, and our continuing efforts to find new mouse models of human disease.

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Support: NIH Common Fund U54 HG006370