Neuropsychopharmacology

ABSTRACTS COLLECTION

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## T1. Evaluating the Effects of C-Reactive Protein Overexpression and Risk for PTSD-Like Behaviors in Mice Exposed to Trauma

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**Background:** Post-traumatic stress disorder (PTSD) remains a growing and often debilitating psychiatric disorder resulting from severe trauma. Increasing evidence suggests a role for systemic and neurological inflammation in the pathophysiology of fear and trauma exposure based psychiatric disorders (Micholpoulos et al., 2017; Haroon et al., 2012). Several large case-control and prospective studies have shown associations with PTSD diagnosis and elevated serum peripheral inflammatory markers, including the acute phase reactant C-reactive protein (CRP) (Spitzer et al., 2010; Passos et al., 2014). Moreover, one prospective study demonstrated that marines who were diagnosed with post-deployment PTSD previously had a two-fold higher baseline serum CRP levels prior to deployment (Eraly et al., 2014). CRP genetic mutations have also been positively correlated with increased PTSD symptoms, further suggesting CRP may confer risk for PTSD pathogenesis (Michopoulos et al., 2015). We tested the hypothesis that increased CRP after gene transfer using an adeno-associated virus (AAV8) encoding murine CRP may confer a higher risk for PTSD-like behaviors after predator stress.

**Methods:** Male C57BL6J mice received a single intrajugular injection of 1011 genome copies (gc) of either AAV8.CRP or AAV8. Null. Four weeks after infection, mice were exposed to either predator stress (10 minutes roomed with a laboratory cat) or handled (stress control). After one week, mice were tested for avoidance behaviors by open field and light-dark box testing. Two weeks post predator stress, we assessed avoidance of trauma cues by measuring exploration of a cue scented with dirty cat litter. Mice where cheek-bled one day after the trauma reminder to measure differential peripheral CRP protein expression post trauma reminder. We further assessed fear conditioning by pairing five separate twenty-second tones (75 dB, 4 kHz) with terminal shocks (0.7 mAmps, 1 second) and forty second inter-trial intervals (ITI). Twenty-four hours later, contextual fear was assessed by exposing mice to the chambers for 16 minutes without tones or shocks. After a final twenty-four hours, mice were exposed again to thirty-two tones (20 seconds, 5 second ITI) within chambers containing altered visual, tactile, and odor dimensions to minimize contextual fear. Following fear conditioning, mice were sacrificed for further brain and peripheral tissue analysis.

**Results:** 6 weeks post AAV8.CRP infection, and subsequent to predator stress and trauma reminder behavioral testing, levels of plasma CRP were almost three-fold higher in animals who had received AAV8.CRP (~18,300 ng/mL, $n = 29$) compared to AAV8. Null (~6,500 ng/mL, $n = 32$, $p < 0.0001$). Predator stress did not significantly alter CRP levels in either group ($n = 17$-19). A composite avoidance score (average of Z-scores across open field, light-dark box, and trauma reminder) demonstrated a main effect of increased avoidance behaviors by two-way ANOVA ($F_{stress} = 9.10$, $p = 0.0036$), though CRP overexpression had no effect either in baseline avoidance or in response to predator stress. Predator stress also increased conditioned fear acquisition as evidence by increased freezing ($F_{stress} = 4.21$, $n = 11$-13, $p = 0.006$), overall there was no effect of either predator stress or CRP on either contextual freezing or in freezing with altered context. CRP expression across peripheral organs and brain will also be discussed.

**Conclusions:** This preliminary study indicated that CRP overexpression exposure did not induce increase risk for avoidance behaviors or conditioned fear after trauma exposure through predator stress, despite high levels of protein. Further investigation is still required to determine if there are sex-specific differences. These data indicate that when limited to adulthood, high CRP loads representing physiological levels in humans do not appear to be sufficient to alter susceptibility to trauma. These results suggest that either CRP gene mutations are required to increase risk or that CRP may only be a marker of immune pathology but is not itself directly contributing to PTSD pathogenesis.

**Keywords:** PTSD, Inflammation, CRP

**Disclosure:** Nothing to disclose.

## T2. Olfactory Memory Codes in the Dentate Gyrus

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**Background:** A central goal of neuroscience is to understand how sensory stimuli are encoded within ensembles of neurons, and how these representations are modified by learning. The hippocampus (HPC) has a well-documented function in encoding spatial information, however, it also encodes non-spatial information crucial for memory formation. Granule cells (GCs) in the

dentate gyrus (DG) of the hippocampus receive input from the lateral entorhinal cortex (LEC), which is known to process olfactory information. This is of interest as these structures are known to be involved in memory generalization, a process impaired in individuals with post-traumatic stress, and are vulnerable to ageing and age-related memory decline. If and how the DG encodes and discriminates olfactory information, and how this information is stored into memory is currently unknown. Here, we identified the DG as a critical hub for generating odor-outcome associations, encoding odor identity using a population code. In addition, we identify how representations change in response to fear learning, defining both stability and dynamism in populations of DG GCs.

**Methods:** We used in vivo 2-photon calcium imaging combined with behavior to determine the mechanisms by which DG GCs represent odor information and how learning alters these representations. We first developed a fear conditioning task in which mice discriminate between three odorants, two similar (methyl butyrate (CS + ) and ethyl butyrate (CS-)) and one distinct (isoamyl acetate (CS-)). After conditioning to CS + , mice are tested to each odor and freezing is assessed to measure how well the mice can discriminate between odors. In a separate context, we performed head-fixed calcium imaging of odor responses in DG GCs in awake mice, before and after odor learning. We blocked synaptic transmission in the LEC-DG pathway using AAV-expressing tetanus toxin light chain (TeLC), and measured effects on learning and neural population odor responses.

**Results:** In both imaging sessions (pre and postconditioning), a subset of DG GCs show odor-selectivity, responding to individual or pairs of odors, as well as odor offset. Analysis of the population code in DG GCs showed high similarity in the ensembles of neurons representing repeated presentations of the same odor, with little overlap in the similarity between separate odors. Using machine learning techniques, we were able to predict odor identity from DG GC activity with very high accuracy ( > 80%) for three odor cues. Moreover, decoding accuracy was just as high in deciphering similar odors as between distinct odor pairs, supporting the notion that the DG is a structure involved in pattern separation. Up to six odors were tested in a given imaging session, and decoding performance remained significant, suggesting DG ensembles can flexibly encode many stimuli and each can be detected as a unique identity. Valence classes were not encoded at the level of DG population responses. Mice were fear conditioned to one of the odors and discrimination scores were taken for freezing to the conditioned odorant and a highly similar odorant. Remarkably, odor classification accuracy before conditioning predicted the animal's behavioral discrimination between similar odor pairs. To determine how neural representations change with learning, we tested two groups of mice, one was conditioned to an odorant with a footshock, and a control group that did not receive a shock, and tracked activity in the same neurons before and after conditioning. We trained our decoder with neural data from the preconditioning session and tested on the data after conditioning. This revealed accurate decoding of all odors across in both groups of mice, indicating that the neural population code for odor identity is stable across time regardless of conditioning. However, changes in single cell responses could be detected in the conditioned animals. We found that the small population of cells tuned to the CS + odor before learning were more likely to maintain their selectivity to the CS + compared to animals that did not receive footshock. In addition, cells tuned to the CS- similar odor in the preconditioning day shifted selectivity toward the CS + odor after conditioning only in the animal receiving a footshock. These results suggest that learning can produce dynamic changes in the small population of selective cells, but the population level response is stable, maintaining odor identity across timescales of days and regardless of conditioning.

**Conclusions:** These results demonstrate a population code for odor identity in an LEC to DG circuit and the stability of that code despite a robust learning event and individual cells altering responsivity. Uncovering the code for odor memories in the DG could offer new insights as to how the DG encodes information and discriminates between incoming information. These studies pave the way to generating new targeted approaches for improving memory generalization deficits often seen in anxiety disorders such as posttraumatic stress or with cognitive decline in ageing.

**Keywords:** In Vivo Calcium Imaging, Dentate Gyrus, Olfactory

**Disclosure:** Nothing to disclose.

### T3. MicroRNA Regulation of Persistent Stress-Enhanced Memory

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**Background:** Mechanisms supporting long-lasting, remote memory are largely unknown, yet highly relevant to neuropsychiatric disorders of memory, such as posttraumatic stress disorder (PTSD). PTSD is a chronic, debilitating disorder in which patients exhibit memories of trauma that are heightened, perseverant and extinction-resistant. Nearly everyone experiences at least one traumatic event in their lifetime, but only 10-20% will later display enduring symptoms of PTSD. We previously developed a stress-enhanced fear learning (SEFL) paradigm in inbred C57BL/6 mice that results in PTSD-like characteristics, including persistently enhanced memory in a subset of mice termed stress stress-susceptible (SS). Importantly, this SEFL protocol allows for the study of molecular phenotypes associated with selective vulnerability, as SS mice can be identified from training data, avoiding mechanistic confounds introduced by additional phenotyping. Relative to stress-resilient animals (SR), SS mice have heightened cFos activation in the basolateral amygdala complex (BLC) upon retrieval of the remote stress memory (30 days post-training) and differential BLC expression of genes with known polymorphisms in human PTSD genomic studies. Disruption of persistent, stress-associated memories is relevant for treating PTSD and microRNAs (miRNAs) are excellent remote memory candidate regulators. miRNAs are endogenous ~20-24 nucleotide RNAs that act as translational repressors through direct binding to the 3'-UTR of target mRNAs and noncleavage degradation of the target mRNA via deadenylation. miRNAs have a wide genomic range of potential target proteins that confers a complexity capable of handling the intricacies of memory but the contribution of miRNAs to long-lasting, remote ( > 30 days) memory is unknown.

**Methods:** We performed small-RNA sequencing and quantitative proteomics on BLC tissue from SEFL animals collected one month after training. Bioinformatic pathway analysis was used to identify candidate miRNAs and target proteins persistently regulated by traumatic memory. In vivo functional manipulation of a candidate miRNA in SEFL animals was performed to further delineate the role of the miRNA in long-lasting memory expression. Levels of the miRNA were measured in postmortem human amygdala tissue with qPCR and in human serum samples from a well-characterized Dutch PTSD military cohort with small-RNA sequencing.

**Results:** We identified persistently changed miRNAs, including mir-135b-5p, and predicted target proteins associated with PTSD-

like heightened fear expression. 18 (24%) of predicted mir-135b-5p protein targets detected in the BLC were differentially regulated between SS and SR animals. Pathway analysis of these 18 mir-135b-5p putative targets that were changed by SEFL identified annotations related to learning and synaptic plasticity. Functional manipulations of BLC mir-135b-5p with short hairpin inhibition or viral-mediated overexpression bidirectionally modulated stress-associated memory in SEFL animals without impacting baseline anxiety levels. mir-135b-5p is expressed in human amygdala and its passenger strand was selectively elevated in members of the military diagnosed with PTSD relative to members without the diagnosis and non-trauma exposed healthy military controls.

**Conclusions:** miR-135b-5p may be an important therapeutic target for dampening persistent, stress-enhanced memory and its passenger strand a potential biomarker for responsivity to a mir-135-based therapeutic.

**Keywords:** Stress Resilience, MicroRNA, Remote Memory

**Disclosure:** Nothing to disclose.

### T4. Precision Medicine and Noradrenergic Biomarkers in PTSD: Preliminary Methods and Validation Data for an Aggregated N-Of-1 Clinical Trial Design Using Pupillometery and Cardiovascular Biomarkers

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**Background:** Posttraumatic stress disorder (PTSD) is a clinical diagnosis that is likely to contain multiple subtypes, with distinct pathophysiological profiles. This unmeasured heterogeneity may contribute to the inconsistent results of pharmacologic treatment trials, where the efficacy of both serotonergic and noradrenergic pharmacotherapies (e.g. SSRI/SNRIs and prazosin) appears to vary both from trial to trial and from person to person. Precision medicine is an approach to clinical research and practice that is designed to address this traditionally unmeasured heterogeneity. Here, we present the preliminary methods and validation data for a new precision medicine trial using pupillometric and cardiovascular biomarkers in PTSD.

Precision medicine seeks to identify patient characteristics that can be measured upfront in order to more effectively match a particular patient to a treatment that is more likely to be efficacious. Previously published post hoc data have suggested that in young males, pre-treatment measurements of cardiovascular biomarkers of peripheral noradrenergic signaling may be able to predict who will have a clinically significant decrease in PTSD symptoms in response to the α1 noradrenergic antagonist prazosin. However, this finding has not been prospectively tested, and is unlikely to generalize to women, older individuals, or those with medical comorbidities. Pupillary dilation, which is also an α1 noradrenaline receptor mediated reflex, may represent an alternative biomarker with wider generalizability.

An additional challenge in precision medicine is that the traditional parallel-group randomized controlled trial is poorly optimized to test hypotheses about predictive biomarkers. They also require that many participants spend the full duration of the study on placebo, which can limit the enrollment of patients with acute symptoms, particularly when the intervention being tested is already widely available. An alternative approach is the use of N-of-1 trial design, where each individual participant spends time in multiple treatment conditions, such as active treatment and placebo; in aggregated N-of-1 trials, a cohort of individuals move through this same type of trial design, and their outcomes are analyzed together to answer questions about e.g. the relationship of a baseline characteristic to the magnitude of treatment response.

Here, we describe the preliminary methods and validation data for a novel aggregated N-of-1 clinical trial designed to test whether pupillometeric and cardiovascular biomarkers predict clinical response to prazosin in treatment-seeking Veterans with PTSD. In addition to preliminary data regarding our biomarkers, we present a set of statistical simulations comparing the power of the selected versus alternative trial designs.

**Methods:** Pupillometric biomarkers measured include maximal dark-adapted pupil diameter, average dilation velocity (ADV) of the pupil following cessation of a brief light pulse, and peak pupillary dilation following phenylephrine ophthalmic application (PPD). Pupillometric biomarkers were measured using a NeurOptics PLR-3000 handheld device. Cardiovascular biomarkers included systolic blood pressure (SBP) measured two minutes after standing and the change in SBP from supine position to seated.

For the statistical simulations of the clinical trial designs, R statistical software was used to simulate the data from: (1) an open-label trial; (2) an open-label phase followed by a blinded discontinuation phase; (3) a traditional crossover trial; and (4) an open label phase, followed by a blinded discontinuation phase and a brief crossover phase. Results were analyzed using a linear mixed effects model.

**Results:** Pupillometric and cardiovascular measurements were well tolerated by participants. In very preliminary results ($N = 3$), but consistent with predictions, total PTSD symptom severity was positively associated with peak pupillary dilation (PPD, $p = .03$), and change in SBP from supine to seated appeared positively associated with ADV ($p = .09$). Trial designs 3 & 4 had substantially higher power with fewer subjects than open label and open label plus blinded discontinuation designs, a pattern maintained across a substantial number of permutations of the modeling assumptions.

**Conclusions:** Assessment of pupillometric and cardiovascular biomarkers is well tolerated, and feasible for a clinical trial or clinical practice setting. The preliminary results from biomarker measurements were consistent with their predicted relationships to PTSD symptom expression. The results of the statistical simulations suggest that both a cross-over trial design, and an aggregated N-of-1 trial design beginning with an open label titration phase, provide superior power over an open label or open label plus discontinuation design in detecting an association between a predictive biomarker and the clinical response to the PTSD pharmacotherapeutic prazosin. These results provide support for a prospective test of whether noradrenergic biomarkers can provide clinically significant predictions of the likelihood of an individual having a meaningful response to prazosin for PTSD, using an aggregated N-of-1 trial design. This framework for approaching precision medicine goals in psychiatric research is expected to have utility for a range of specific research topics.

**Keywords:** PTSD, Noradrenaline, Clinical Trial Design, Biomarkers, Prazosin

**Disclosure:** Nothing to disclose.

### T5. Unconditioned Responses During a Fear-Conditioning Paradigm Predict Physiological and Neural Correlates of Fear Extinction Recall in Anxious Individuals

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**Background:** Fear conditioning and extinction paradigms are used in the laboratory to better understand how individuals learn fear and safety associations. In this type of paradigm, a neutral stimulus (e.g., a colored lamp, conditioned stimulus, CS + ) is presented along with an aversive stimulus (e.g., shock), which in itself provokes a physiological reaction (named unconditioned response, UCR). Eventually, a similar physiological response can be induced with the presentation of the CS + alone, what is called a conditioned response. Following this fear-conditioning phase, extinction learning takes place where the CS + is presented multiple times without any reinforcement, which gradually decreases the physiological reaction. Extinction recall memory also be tested after a delay in order to examine whether the safety memory trace, formed during extinction learning, has been properly consolidated and/or can be adequately retrieved. Previous studies have shown that individuals suffering from post-traumatic stress disorder (PTSD) and from anxiety disorders exhibit deficient extinction recall, either at the neural and/or physiological level. This being said, beyond the identified group differences, there is a need to identify biomarkers that could be used as predictors of extinction recall. Here, we tested whether UCRs to an aversive stimulus during fear learning could predict psychophysiological and neural responses during extinction learning and extinction recall.

**Methods:** Eighty-seven individuals suffering from an anxiety disorder were recruited and exposed to a 2-day fear conditioning and extinction protocol in a fMRI environment. On day 1, three colored lamps were presented, two of which were partially reinforced with a mild electric shock (CS + ) and the third one was never reinforced (CS-). Immediately after, extinction learning took place where one of the CS + was presented along with the CS-. The next day, extinction recall was tested by presenting all three cues again. For each phase, skin conductance responses (SCR) were measured. UCRs to the shock presentations were computed for each individual. Four groups were generated based on their UCRs (from very low to very high UCRs). Between-groups comparisons were tested for skin conductance responses and for BOLD signal during early and late extinction learning and during early extinction recall. For each fMRI contrast mentioned above, we looked for a main effect of group in the following regions of interests (ROIs) known to be involved in the fear circuitry: amygdala, hippocampus, insular cortex, dorsal anterior cingulate cortex (dACC), and ventromedial prefrontal cortex. Significant clusters ($p < 0.005$) identified within the masks (based on ROIs) were tested for small-volume corrections. Beta-weights were extracted in order to conduct post-hoc analyses.

**Results:** Groups did not differ in terms of education, shock intensity, and sex distribution. They did however differ on age. Therefore, all analyses presented below controlled for that variable. For extinction learning, a significant Time X Group interaction was found for SCR, $F(3,70) = 3.32$, $p = 0.025$. Decomposing the interaction, we found that the group exhibiting the highest UCR during fear conditioning had higher SCR to CS + during early extinction learning relative to all other groups. Importantly, no group differences were found during late extinction learning ($p = 0.74$), suggesting that all groups reached similar physiological fear levels at the end of extinction learning. The fMRI analyses yielded no significant group differences in the ROIs. For extinction recall, a Stimulus X Group interaction was revealed, $F(3,67) = 3.98$, $p = 0.011$, with the group with the highest UCR exhibiting higher fear levels to the CS + relative to the other groups. No group effects were found for the CS-. Moreover, a significant correlation was revealed between UCR and SCR to the CS + during extinction recall ($r = 0.499$, $p < 0.001$). At the neural level, a main effect of Group was found in the dorsal anterior cingulate cortex ($pfwe = 0.048$). Post-hoc analyses on the extracted beta-weights revealed lower dACC activation in the group with the lowest UCR relative to the group exhibiting the highest UCR ($p = 0.017$).

**Conclusions:** Our results suggest that unconditioned responses, assessed via skin conductance responses, during a fear-learning paradigm could inform about fear reactivity during extinction recall, assessed both with physiological and neural measures. Importantly, this result is specific to extinction recall, given that all groups had similar fear levels, in terms of SCR and BOLD signal, at the end of extinction learning. Given that deficits in extinction recall have been shown to characterize multiple fear-based and anxiety disorders, these results point to a potential biomarker that could be used to predict later expression of fear. Future studies in anxiety patients should assess whether unconditioned responses could be predictive of response to exposure-based therapy, which relies on fear extinction learning principles.

**Keywords:** Unconditioned Responses, Fear Extinction, Extinction Memory Recall, Skin Conductance Responses, Anxiety Disorders

**Disclosure:** Nothing to disclose.

## T6. Neural Mechanisms of Spatial Attention Deficits in Trauma

Abstract not included.

## T7. Longitudinal Investigation of Advanced Epigenetic Age and Change in Peripheral Biomarkers of Inflammation and Metabolic Syndrome

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**Background:** Epigenetic age estimations based on DNA methylation (DNAm) profiles can be used to predict human chronological age with a high level of accuracy. These DNAm age profiles can also be used to index advanced cellular age, when estimated DNAm age exceeds chronological age. The goal of the present study was to understand the biological changes associated with advanced epigenetic age over time, given that accelerated cellular age has previously demonstrated association with a variety of diseases and early death.

**Methods:** We investigated associations between two measures of epigenetic age acceleration (Hannum and Horvath) and changes in peripheral metabolic and inflammatory markers in a longitudinal cohort of 179 veterans who were assessed over the course of two years. The cohort was enriched for PTSD and other psychopathology and we have previously demonstrated that trauma-related psychopathology is associated with advanced epigenetic age (Wolf et al., 2016), making this cohort particularly relevant for understanding the biological correlates of advanced epigenetic age over time.

**Results:** Analyses revealed that, controlling for chronological age, sex, race, education, PTSD, and baseline biomarkers, advanced DNAm age at baseline was associated with residualized change in metabolic syndrome (MetS) severity at T2, suggestive of worsening MetS pathology ($p < .001$). Follow-up analyses found that this association was specific to worsening obesity (as measured by lipid panels and indicators of abdominal obesity: $p = .001$). Advanced Hannum DNAm age at T1 was nominally associated with residualized change in C-reactive protein (CRP) levels and total white blood cells at T2 (for CRP: $p = .058$; for white blood cells: $p = .051$). We also found that PTSD symptom severity at T1 predicted decreasing (i.e., worsening) CD4/CD8 T-cell ratios

at T2 ($p = .015$). There were no significant associations between baseline Horvath DNAm age and change in any of the biological variables at T2.

**Conclusions:** Results suggest that advanced epigenetic age may hasten pathological metabolic and inflammatory processes, which could be one mechanism linking advanced epigenetic age to morbidity and mortality.

**Keywords:** DNA Methylation, Metabolic Syndrome, C-Reactive Protein, PTSD

**Disclosure:** Nothing to disclose.

### T8. Functional Impacts of Acute Stress on Negative Affective Circuit Function in Anxiety and Depression

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**Background:** Dysfunction in the hypothalamic-pituitary-adrenal (HPA) axis and negative affective circuit, which includes the amygdala and prefrontal cortex (PFC), have independently been associated with mood and anxiety disorders. Consistent with these relationships, both disorders are conceptualized within a vulnerability-stress framework in which stress is thought to cause long-term alterations in the function of affective circuitry which in turn, sensitizes the stress response. Threat-elicited hyper-activity of the amygdala along with hypo-activity of the prefrontal cortex has been observed in individuals with mood and anxiety disorders and with high-trait anxiety. Mood and anxiety disorders have also been associated with HPA dysfunction assayed by diurnal cortisol profiles (most consistently elevated), and by abnormal negative feedback of stress hormones indexed by the dexamethasone suppression test. HPA stress responses are modulated by input from the amygdala and PFC. Conversely, baseline levels of stress hormones (including cortisol) as well as the release of stress hormones in response to stressors are also both associated with amygdala and PFC reactivity and connectivity. Despite the above evidence of abnormal negative affective circuit profiles in nodes such as the amygdala and PFC that are critically involved in and influenced by stress responding, it remains unexplored how HPA dysregulation may contribute to and/or explain these neuroimaging phenotypes. Addressing this issue, we tested the contribution of abnormal stress responding in the negative affective circuit and clinical profiles by using a repeated-measures design in which a commonly used MRI stress induction paradigm is interleaved between emotional face probes, in combination with cortisol assays.

**Methods:** 16 unmedicated individuals experiencing anxiety and depression symptoms completed the same standardized imaging procedure twice under two conditions (stress induction, control) separated by at least once week. Negative affective circuit function was assessed using an established emotional face task. Regions of interest (ROIs) included bilateral amygdala and the medial PFC (mPFC). During the stress visit, the emotion task was preceded by a stress induction paradigm (Montreal Neuroimaging Stress Task). In contrast, on the control visit, the emotion task was preceded by a neutral control task. Subjective stress and cortisol levels were acquired at multiple time points across the imaging sessions. Cortisol trajectories were visually inspected and used to classify participants into those who did and did not have the expected stress response following induction. Mean contrast estimates for the anger versus neutral face contrast were extracted for each of the ROIs and entered into subsequent analyses. Linear mixed models were then used to assess the impact of condition (stress induction, control) and stress response status (stress response, no stress response) on negative affective circuit function.

**Results:** As a group, the stress induction significantly increased cortisol levels as measured by the area under the curve ($t = 2.43$; $p = 0.028$). However, there was significant variation across individuals: six participants elicited the expected cortisol stress response while ten did not. Negative affective circuit function did not differ between the two conditions (all p's = > 0.05) nor between the stress response status groups (all p's > 0.05). Importantly, there was a significant interaction between condition and stress response status for amygdala activation (left: $F = 10.22$, $p = 0.006$; Right: $F = 5.93$; $p = 0.029$). Those who elicited a cortisol stress response following the stress induction also saw an increase in amygdala activation across conditions (left: $t = 2.28$, $p = 0.039$; Right: $t = 1.96$, $p = 0.060$). Conversely, those who did not elicit a cortisol response saw a decrease in amygdala activation across conditions (left: $t = -2.28$, $p = 0.039$; Right: $t = -1.44$, $p = 0.022$). No effects were found for the PFC (all $p > 0.05$).

**Conclusions:** Our results advance our understanding of how HPA axis dysfunction may be associated with abnormal negative affect circuit function in anxious and depressed individuals.

**Keywords:** Amygdala, HPA Axis, Cortisol, Anxiety, Depression

**Disclosure:** Nothing to disclose.

### T9. Childhood Maltreatment is Associated With White Matter Alterations in Temporal Pathways Regardless of PTSD Diagnostic Status: A Probabilistic Tractography Analysis

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**Background:** Consistent with a large body of literature demonstrating white matter tract abnormalities including reduced fractional anisotropy (FA) in temporal tracts in posttraumatic stress disorder (PTSD), our group previously found reduced FA in the left inferior longitudinal fasciculus (ILF) in PTSD patients compared with trauma-exposed controls (Olson et al., 2017). However, because childhood maltreatment and PTSD diagnosis were statistically overlapping, it was unclear whether those group differences could be attributable to childhood adverse experiences. Choi et al (2012) previously found that witnessing inter-parental violence was associated with reduced FA in the left ILF, a part of the visual limbic pathway involved in processing visual and auditory stimuli related to emotion, learning, and memory. They theorized that exposure to threatening facial expressions and voices may result in an over-activation of visual-limbic cortices connected by the ILF, so that repeated exposure to violence ultimately inhibits the development of this pathway. Given these findings, we attempted to replicate our study demonstrating reduced FA in the ILF in PTSD, in a new sample including controls reporting a broad range of childhood adverse event exposure. We hypothesized that reduced FA would be associated with PTSD diagnostic status and also with total exposure to childhood maltreatment. Previous studies have demonstrated that forms of maltreatment including emotional abuse and emotional/physical neglect that do not meet DSM-IV criterion A for PTSD are also associated with white matter tract abnormalities. Therefore, we hypothesized that non-criterion A childhood maltreatment (emotional abuse, emotional and physical neglect) would yield similar effects on ILF FA as forms of childhood abuse that are consistent with criterion A exposure (physical and sexual abuse).

**Methods:** This sample included 93 participants, age 20-50 (M = 33.50, SD = 8.37): 32 with DSM-IV lifetime PTSD (19 F, 13 M), 27 trauma-exposed non-PTSD controls (TENC: 15 F, 12 M), and 34 healthy controls (17 F, 17 M). Participants completed the Structured Clinical Interview for DSM-IV (SCID-IV I/P), the Clinician Administered PTSD Scale (CAPS) and the Childhood Trauma Questionnaire (CTQ). The CTQ generates scores on 5 subscales (physical abuse, sexual abuse, emotional abuse, emotional neglect, and physical neglect). Scores were summed to generate a total abuse exposure variable. We also summed the physical and sexual abuse categories to create a combined criterion-A-type exposure score and summed the neglect and emotional abuse categories to create a combined non-criterion-A-type exposure score. Diffusion tensor imaging (DTI) was collected on a Siemens 3.0 T Trio (TR, 7300 ms; TE; 80 ms; 72 directions at b = 1000 plus 8 b = 0 images). Data were preprocessed in FSL and automatic probabilistic tractography was performed using FreeSurfer's TRACULA to measure DTI metrics (FA, mean diffusivity (MD), axial diffusivity (AD), radial diffusivity (RD)) within the ILF bilaterally.

**Results:** In contrast to our previous report, after controlling for age and sex, there was no significant effect of diagnostic status on ILF FA, $F(4,176) = 1.107$, $p = 0.355$, partial eta squared = 0.025. Across the entire sample, after controlling for age and sex, greater total childhood trauma exposure was associated with reduced ILF FA, $F(2,87) = 3.656$, $p = 0.030$, partial eta squared = 0.078. This effect was significant bilaterally (left ILF, $F(1,88) = 6.788$, $p = 0.011$, partial eta squared = 0.072; right ILF, $F(1,88) = 5.350$, $p = 0.023$, partial eta squared = 0.057). The association between childhood trauma exposure and reduced ILF FA persisted after additionally controlling for diagnostic group, $F(2,85) = 4.118$, $p = 0.020$. The effect of childhood maltreatment on ILF FA was significant for criterion-A-type exposures (i.e., physical and sexual abuse), $F(2,87) = 3.230$, $p = 0.044$, as well as for non-criterion-A-type exposures (i.e., emotional abuse and emotional and physical neglect), $F(2,88) = 3.519$, $p = 0.034$. The effect of total childhood trauma exposure also was significantly associated with reduced ILF FA within the combined TENC and PTSD groups, $F(2,53) = 3.545$, $p = 0.036$. Despite associations between FA and childhood trauma exposure, childhood trauma was not associated with alterations in the other DTI metrics (MD, RD, AD).

**Conclusions:** Total exposure to childhood maltreatment was associated with reduced FA in both left and right ILF independent of PTSD diagnostic status. Furthermore, both criterion A type maltreatment and non-criterion A type maltreatment were correlated with reduced FA in this tract. In contrast to our previous report, these results suggest that differences in the structural integrity of white matter in the ILF may be due to exposure to childhood maltreatment rather than diagnostic status. Considering the involvement of the ILF in processing visual and auditory information, exposure to adverse early life events may be implicated in abnormal sensory processing. Our results suggest that reduced ILF FA (and possibly associated sensory processing abnormalities) is associated with childhood maltreatment broadly, including neglect and emotional abuse as well as more violent forms of exposure.

**Keywords:** PTSD, Diffusion Tensor Imaging (DTI), Childhood Maltreatment, Child Abuse and Neglect

**Disclosure:** Nothing to disclose.

### T10. The Role of Respiratory Noradrenergic Projections to the Basal Lateral Amygdala in Anxiety-Related Behavior

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**Background:** The most commonly reported symptom of panic attacks is respiratory dysfunction, such as hyperventilation. It has been shown that subsets of patients with anxiety disorders have a greater sensitivity for carbon dioxide level imbalances, and exposure to high levels of carbon dioxide (hypercapnia) can induce anxiety-like behavior, such as panic attacks. The false suffocation alarm hypothesis posits that inappropriate activation of respiratory chemoreceptors, sensors of carbon dioxide levels, in response to benign indices triggers an alarm of suffocation danger and may contribute to anxiety and panic disorders. Two potential candidates that may link anxiogenic and respiratory circuitry are the central noradrenergic (NA) system and the basal lateral amygdala (BLA), both of which have been shown to play roles in both breathing and anxiety. Therefore, we hypothesize that projections from the brainstem NA nuclei to the BLA play a functional role in modulating the hypercapnic ventilatory response and anxiety-like behavior in mice.

**Methods:** To test this hypothesis, we targeted NA neurons that project to the BLA by using a Cre-expressing retrograde virus applied to the BLA of our intersectional DBH-p2a-FLPo; RR5, multicolor reporter line to conduct projection mapping of NA neurons that project to the BLA and DBH-p2a-FLPo; RR1 and RR2 DREADD mice to specifically inhibit or stimulate, respectively, NA neurons that project to the BLA in vivo. In these mice, we measured respiratory output using whole-body plethysmography and anxiety-like behavior using open field, light dark and elevated plus maze.

**Results:** Anatomical projection mapping reveal that NA neurons that project to the BLA are highly collateralized, projecting to additional brainstem and forebrain regions associated with respiration and negative affective disorders, and that these NA projections are primarily derived from the locus coeruleus (LC). Upon stimulation of NA neurons that project to the BLA, there was no change in hypercapnic response ($n = 9$, $p > 0.5$). However, we observed increased distance traveled in the center ($n = 9$, $p < 0.5$), decreased distance traveled in the perimeter ($n = 9$, $p < 0.5$) and decreased latency to enter the center of the open field. Additionally, there was decreased distance traveled in the dark zone of the light dark assay ($n = 9$, $p < 0.5$), but no change in anxiety-related behavior in elevated plus maze. Conversely, upon inhibition of NA neurons that project to the BLA, there was a decrease in the hypercapnic response ($n = 7$, $p > 0.5$), although not significant. We observed a decrease in distance traveled in the center of the open field ($n = 7$, $p < 0.5$) and an increased distance traveled in the illuminated region of the light dark assay ($n = 7$, $p < 0.5$). There was also decreased distance traveled in the enclosed arms ($n = 7$, $p < 0.5$), as well as increased latency to first entry of enclosed arms in the elevated plus maze ($n = 7$, $p < 0.5$). Respiratory data was analyzed using a linear mixed-effects regression model, significance at an alpha = 0.05. Behavior data was analyzed using multiple t-tests, assuming samples from populations with same scatter (SD) and corrected using Holm-Sidak method, significance at an alpha = 0.05.

**Conclusions:** Our preliminary results suggest that NA neurons that project to the BLA may play a role in both the hypercapnic response and anxiety-related behavior, beginning to illustrate the role of respiratory circuitry in negative affective disorders, suggesting that further study of NA subpopulations based on efferent target should greatly advance our current understanding of related psychopathologies.

**Keywords:** Amygdala, Noradrenaline, Anxiety, Respiration, DREADD Receptor

**Disclosure:** Nothing to disclose.

**T11. Open Board**

**T12. Determining the Role of the Central Amygdala in Modulating Complex Fear States**

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**Background:** Anxiety disorders are debilitating psychiatric illnesses that are characterized by maladaptive responses to perceived threat. Threatening stimuli cause alterations in neuronal circuits that act to elicit adaptive defensive responses. Importantly, associative learning causes plastic changes in these neuronal circuits that allow organisms to use predictive cues to adequately respond to danger. Deficits or abnormalities in the ability to express appropriate defensive behavior in response to learned cues could underlie anxiety disorders such as specific phobia, panic, and post-traumatic stress disorder; however, the neuronal processes that regulate defensive response intensity are poorly understood. Associative learning related to aversive experience is modeled in the laboratory with Pavlovian fear conditioning. Freezing is the dominant behavioral response elicited in this paradigm and has therefore become the standard experimental readout of aversive learning. However, defensive responses are highly complex and there is a critical need to move beyond reductionist readouts of learned fear towards methods that capture the full range of behavioral adaptations to perceived threat. Indeed, we need to establish a better understanding of the neurophysiological changes that drive defensive responding if we are to develop better therapeutics.

**Methods:** We recently developed an associative learning paradigm in which auditory stimuli elicit scalable intensities of defensive behavior in a context-dependent manner. We are using this conditioned flight paradigm to establish robust behavioral profiles of different fear states as well as investigations into the neurophysiological underpinnings of complex responding. Our hypothesis is that the central nucleus of the amygdala (CEA) is a critical site that controls the intensity of defensive responses.

We are utilizing extracellular recordings in freely moving mice to record the activity patterns of CEA neuronal populations. We are also using a "phototagging" approach in which opsins are expressed in specific populations of genetically defined CEA neurons to allow for optogenetic identification of recorded neurons. These approaches allow us to obtain temporally precise correlations of neurophysiology with defined fear states, with a primary goal of identifying activity patterns that predict shifts in defensive responding.

**Results:** To date, we have recorded 177 CEA neurons in 19 mice subjected to the conditioned flight paradigm. Approximately 20% of these units are response specifically to the footshock (the unconditioned stimulus, US), 50% are responsive to conditioned auditory stimuli (CSs), and 30% are unresponsive to either the US or CSs. Among the CS-responsive neurons, 30% respond to the cue that drives flight and 20% respond to the cue that drives freezing. There were also populations of neurons that were inhibited by each of the CSs. Among the US-responsive units, we found two main categories of response – transient ($< 3$ s) and sustained ($> 3$ s), with both populations containing subpopulations with excitatory or inhibitory responses.

Using the context-dependence of the flight paradigm, we have also tracked the activity of CeL-ON and CeL-OFF neurons in high and low fear states. We find that CeL-OFF neurons ($n = 9$) are strongly inhibited throughout the flight paradigm. Cel-ON neurons ($n = 6$), however, are excited during the CS eliciting freezing and inhibited by the CS inducing flight, but only in the flight context. Finally, using the phototagging approach in PKCδ-Cre mice, we found that 5/10 PKCδ neurons could be identified as CeL-OFF. These PKCδ + CeL-OFF neurons were neither excited nor inhibited during the conditioned flight paradigm.

**Conclusions:** The proposed experiments are providing vital information about the neurobiological basis of complex fear states. We are currently performing analyses aimed at identifying neuronal populations with activity that is predictive of switching between high and low fear states. We believe that this will build a foundation for identifying and verifying novel therapeutic targets aimed at improving understanding and treatment of the brain dysfunction underlying debilitating mental disorders that negatively affect the lives of millions.

**Keywords:** Fear, Amygdala, Electrophysiology, Anxiety Disorders, Central Amygdala

**Disclosure:** Nothing to disclose.

**T13. Parallel Circuits From the Bed Nuclei of Stria Terminalis to the Lateral Hypothalamus Drive Opposing Emotional States**

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**Background:** The mechanisms underlying aversion and reward are beginning to be uncovered, with substantial efforts focused on the intricate connectivity of the lateral hypothalamus (LH). LH neurons containing the neuropeptide hypocretin (Hcrt; orexin) modulate affective components of arousal, yet the precise synaptic inputs linking Hcrt-LH activity and emotional behavior remain to be fully defined. We set out to uncover how Hcrt-LH neurons respond to ethologically salient stimuli, generate hedonically-valenced behavioral states, and integrate diverse synaptic inputs from specific upstream cell types.

**Methods:** Here, we used modified rabies monosynaptic input mapping to identify major sources of long-range input onto Hcrt-LH neurons originating from neuronal populations in the bed nuclei of stria terminalis (BNST; a heterogeneous structure of the extended amygdala). To functionally interrogate BNST--> LH circuitry, we used viral, optical, chemogenetic, and slice electrophysiological methods for monitoring and manipulating neural activity with genetically-defined and pathway-specific resolution in mice.

**Results:** Specifically, we characterized two non-overlapping and spatially-segregated GABAergic BNST cell groups that both exhibit axonal projections to the LH, but express distinct neuropeptide markers (corticotropin-releasing factor, Crf; and cholecystokinin, Cck). We found that Crf-BNST and Cck-BNST neurons provide differentially abundant synaptic inputs onto Hcrt-LH neurons, display discrete physiological responses to salient stimuli, drive opposite emotionally-valenced behaviors, are differentially required for appetitive behavioral approach, and receive synaptic inputs from unique upstream neural networks.

**Conclusions:** Together, the data provide an advanced model for how parallel genetically-defined BNST--> LH pathways promote divergent emotional states via distinct connectivity patterns of circuit-specific neuronal subpopulations. Our novel findings suggest a mechanistic framework for BNST--> LH circuit dysregulations in psychiatric disorders and will therefore be broadly relevant to the fields of neuroscience and mental health, as future studies will inform development of improved therapeutic approaches.

**Keywords:** Lateral Hypothalamus, Extended Amygdala, Hypocretin, Orexin, CRF

**Disclosure:** Nothing to disclose.

## T14. Epigenetic Programming of Chromatin Accessibility by Stress During Puberty

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**Background:** Adverse childhood experiences are one of the greatest predictors for affective disorder presentation for women. However, few animal models exist that address female-specific risk factors or unique periods across the lifespan. As the pubertal transition is marked by dynamic hormonal changes and ensuing reorganization of the brain, it represents a window of sex-specific vulnerability to adverse experiences. Periods of hormonal flux in the female lifespan, including pregnancy, exacerbate the risk for affective disturbances and promote stress dysregulation, a key feature of affective disorders. We have established a translationally relevant mouse model in which pubertal adversity leads to broad stress dysregulation in adulthood that is dependent upon hormonal status. Our previous work in humans and mice has shown that increases in allopregnanolone are necessary to produce the blunted HPA axis response in stressed females. Allopregnanolone likely acts on a reprogrammed GABA system within the paraventricular nucleus of the hypothalamus (PVN), as RNA-Seq analysis of the PVN during pregnancy revealed alterations to GABA system gene expression by pubertal stress. However, it is unclear what is responsible for long-term reprogramming of the GABA system. Prior RNA-Seq also revealed that females stressed during puberty had increased expression of a host of immediate early genes at baseline during pregnancy. Immediate early gene expression requires that their promoters be accessible to the intracellular cascades that initiate their transcription. Therefore, we hypothesize that, even at baseline, the chromatin in the PVN of pubertally stressed females is in a more open, permissive state.

**Methods:** Female mice were exposed to chronic variable stress from postnatal days 21-34. At 10-12 weeks old, females were sacrificed, either in virgin or pregnant state. Brains were frozen until the PVN could be extracted. PVN tissue was subject to ATAC-Sequencing, a technique which allows for the direct assessment of chromatin openness and interrogation of which genes are available for transcription, to assess pubertal stress-induced epigenetic programming.

**Results:** ATAC-Seq signal intensity in the PVN was assessed for effects of pubertal stress and hormonal state, allowing for the identification of alterations to the chromatin accessibility landscape by pubertal stress, pregnancy, or requiring both. Rigorous quality analysis was done to account for factors such as duplicate reads and mitochondrial reads that can impact analysis.

**Conclusions:** Together, these studies support a role for long-term alterations in chromatin accessibility in the PVN in the stress dysregulation phenotype that is observed only in adult female mice that had both experienced stress during puberty and were pregnant. This translationally-relevant mouse model provides the opportunity to understand the molecular underpinnings of risk for stress dysregulation, a central endophenotype of affective disorders.

**Keywords:** GABA, Allopregnanolone, Chromatin, ATAC-seq, Stress Models

**Disclosure:** Nothing to disclose.

## T15. Increase GABAergic Neurons in Brain Organoids Derived From Children With Autism Spectrum Disorder

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**Background:** Aberrant gamma-aminobutyric acid (GABA) system, the major inhibitory neurotransmitter in the CNS, is highly implicated in autism spectrum disorder (ASD). Aberrant gamma-aminobutyric acid (GABA) system, the major inhibitory neurotransmitter in the CNS, is highly implicated in autism spectrum disorder (ASD). However, mechanisms are essentially unknown.

**Methods:** To study the proposed questions, we reprogram fibroblasts that are terminally differentiated back to pluripotent stem cells (iPSCs) allows the investigation of human neurodevelopment in vitro. The iPSCs are developed into three-dimensional organoids that resemble human brain tissue.

**Results:** We found that control non-affected organoids show neuronal differentiation potential with cortical and cell type-specific markers and display functional voltage-gated channels and neuronal excitability. Moreover, qPCR and immunocytochemistry concomitantly suggest the increase of GABAergic neuronal markers in ASD organoids, such as DLX, vGAT and GAD67.

**Conclusions:** We conclude that there is an increased GABAergic neuronal fate in cortical organoids derived from children with ASD.

**Keywords:** Autism, GABA, Brain Organoids

**Disclosure:** Nothing to disclose.

## T16. Neural Abnormalities During Successful and Failed Stopping in Attention Deficit/Hyperactivity Disorder: A Meta-Analysis Using Whole-Brain Statistical Parametric Maps

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**Background:** Patients with Attention Deficit/Hyperactivity Disorder (ADHD) often demonstrate abnormalities in brain functioning during tasks of inhibitory control. However, most individual neuroimaging studies have been small. Moreover, previous meta-analyses of functional magnetic imaging studies (fMRI) of inhibitory control in ADHD have the following limitations: (i) they have been based on peak-coordinates rather than whole-brain data; (ii) they have not considered behavioral performance; (iii) they have focused on between-group findings and have not observed within-group patterns of activation/deactivation; (iv) they have included data from different inhibitory tasks and used multiple contrasts; (v) and they did not examine both successful and failed inhibition. To overcome these limitations, we thus meta-analyzed whole-brain and task performance data from 10 studies that used the same probe of successful and failed inhibitory processes (the stop signal task).

**Methods:** A preliminary meta-analysis ($n = 834$) was performed on 10 studies comparing patients with ADHD ($n = 379$; age range $= 8$-$50$, 269 males) and controls ($n = 455$; age range $= 8$-$50$, 273 males) on the stop signal task using event-related fMRI. Performance data was analyzed using a random-effects meta-analysis implemented in the Metafor package for R. Seed-based d Mapping was used to perform within-group and between-group fMRI meta-analyses based on whole-brain statistical parametric maps from the original studies. The examined contrasts were successful-stop > go and failed-stop > go. The protocol was registered with PROSPERO (CRD42018095365).

**Results:** There was no significant group difference in mean stop signal reaction time ($d = 0.24$ $p = .11$). During successful stopping, patients with ADHD showed underactivation relative to controls in

left inferior frontal gyrus (IFG)/orbitofrontal cortex (OFC), left amygdala and bilateral thalamus/right caudate, but reduced deactivation relative to controls in posterior cingulate cortex (PCC). During failed stopping, patients showed underactivation in dorsomedial prefrontal cortex and left IFG/OFC/anterior insula, but reduced deactivation in PCC and bilateral putamen (all SDM-Z > 2, $p < .005$).

**Conclusions:** During both successful and failed stopping, patients with ADHD showed reduced activation in the predominantly left hemisphere salience network (SN) and reduced deactivation in posterior default mode network (DMN). The SN has been implicated in shifts between DMN and task positive networks that follow upon the detection of stimuli which indicate the need for greater externally focused attention and executive control. Thus, our central finding of greater DMN and reduced left SN activation provides further evidence that patients have deficits in performing dynamic adjustments between interoceptive and exteroceptive attention. In unaffected controls, deactivation within dopamine-innervated dorsal striatum during failed stopping has been hypothesized to represent a learning signal that guides improvements to future performance. Failures of deactivation within bilateral putamen in ADHD are therefore in line with recent suggestions of impaired dopaminergic functioning and prediction error signaling in the disorder.

**Keywords:** ADHD, Response Inhibition, Functional MRI (fMRI), Stopping, Meta-Analysis

**Disclosure:** Nothing to disclose.

### T17. Neurocognitive Correlates of Resilience in Youth With a History of Traumatic Stress Exposure

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**Background:** Children and adolescents often grow up experiencing significant stress at a sensitive period of brain development. For some susceptible individuals, this stress exposure is associated with increased risk for mental health disorders, especially depression. Yet many individuals exposed to traumatic stress do not show significant psychopathology. These individuals are often characterized as resilient. Significant research has been dedicated to identifying risk factors and studying mechanisms of developmental psychopathology, but there is limited understanding of the mechanisms that underpin resilience in developing humans. We have recently reported detrimental clinical and cognitive correlates of childhood trauma exposure in the Philadelphia Neurodevelopmental Cohort (PNC). The PNC includes ~9,500 genotyped community youths (ages 8-21), some with significant trauma exposure, who have been robustly phenotyped for psychopathology and neurocognitive functioning. In this study, we aimed to identify neurocognitive measures that are associated with resilience in PNC youth.

**Methods:** Traumatic stress exposure was assessed through screening of eight potentially traumatic events that include situations in which the participant (1) experienced a natural disaster; (2) experienced a bad accident; (3) thought that s/he or someone close to him/her could be killed or hurt badly; (4) witnessed someone getting killed, badly beaten, or die; (5) saw a dead body; or if s/he ever was a victim of one of the following assaults: (6) attacked or badly beaten, (7) threatened with a weapon, or (8) sexually forced (including but not limited to rape). Psychopathology was assessed using a mood-anxiety factor score, calculated using factor analysis of item-wise (i.e. symptom-level) psychopathology responses from clinical assessment. Neurocognitive functioning was evaluated by the 1-hour Penn Computerized Neurocognitive Battery (CNB). The CNB includes multiple tests assessing 4 cognitive domains: 1) executive function; 2) episodic memory; 3) complex reasoning; 4) social cognition. To identify correlates of resilience, we employed a $2 \times 2$ factorial design: high/low traumatic stress exposure; high/low psychopathology. Resilient individuals represent youths with high traumatic stress exposure and low psychopathology. Associations among trauma exposure, psychopathology, and their interaction (independent variables) with neurocognitive functioning (dependent variable) were studied using linear regression models controlling for age, sex, and socioeconomic status.

**Results:** In youths with high traumatic stress exposure, those with low psychopathology (resilient, $N = 732$) showed superior overall cognitive efficiency compared to those with high psychopathology ($N = 1595$), while no such differences were observed comparing high and low psychopathology in low traumatic stress exposure individuals (exposure X psychopathology interaction $t = 2.274$, $p = .023$). Follow-up analyses to break down overall cognitive efficiency to specific cognitive domains revealed a similar pattern in complex reasoning efficiency (exposure X psychopathology interaction $t = 2.168$, $p = .03$), with no significant interactions in other cognitive domains.

**Conclusions:** Resilient youths who exhibit low mood-anxiety psychopathology in the face of high traumatic stress exposure show a distinct neurocognitive efficiency pattern that is characterized by superior complex reasoning, independent of sex, age, and socioeconomic status. While a cross-sectional design does not allow causal inference, results may shed light on specific cognitive mechanisms underpinning resilience.

**Keywords:** Risk and Resilience, Childhood Trauma, Cognitive Functioning, Developmental Psychopathology

**Disclosure:** Taliaz Health, Advisory Board, Taliaz Health, Stock / Equity

### T18. The Importance of Prefrontal Regions in Frustration Tolerance in Children With ADHD

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**Background:** Emotion dysregulation, particularly low frustration tolerance, is a key impairment in children with Attention-Deficit/Hyperactivity Disorder (ADHD). Observational and behavioral studies of frustration tolerance in children with ADHD have shown that compared to typically-developing (TD) children, children with ADHD show greater levels of frustration, are more likely to quit a frustrating task, exhibit greater attention to the negative aspects of a task and display less constructive patterns of emotional coping when frustrated. Taken together, this literature stresses the clinical importance of elucidating the mechanisms underlying poor frustration tolerance in children with ADHD.

However, to date, few studies have examined the neural mechanisms of frustration in children with ADHD. Frustration has been theorized to involve: (1) core limbic and reward regions (amygdala [AMG], orbitofrontal cortex [OFC], ventral striatum) involved in the assessment of emotional/reward salience and generation of emotion responses; (2) frontal cortical (dorsolateral prefrontal cortex [dlPFC]) and dorsal striatal regions involved in the cognitive control of emotional responses, and (3) regions involved in the interface between emotional and cognitive control circuitry (medial prefrontal cortex [mPFC] and anterior cingulate cortex [ACC]).

The objective of this study is to examine the relationship between cortical and subcortical regions of interest (ROIs) and frustration using a novel behavioral paradigm in children with ADHD with and without comorbidity compared to TD children.

**Methods:** Participants included 102, 8-12 y.o. children: typically-developing controls (TD, n = 38), children diagnosed with DSM-5 ADHD (ADHD, n = 32) or children diagnosed with ADHD plus a comorbidity including Oppositional Defiant Disorder, anxiety or depressive disorders (Comorbid, n = 32).

Participants completed a novel Frustration Go/No Go paradigm with performance-based incentives delivered across three blocks including a "frustrative" second block involving 50% "rigged" Go trials in which the stimuli did not move to the target. To assess frustration during the task, participants completed affect ratings at four points (baseline and after each block).

High resolution MPRAGE images were acquired for all participants. Frontal lobe volumes were extracted using an atlas developed by our Center and employed within FreeSurfer including: dlPFC, ACC, OFC, and mPFC. Subcortical volumes were extracted using a validated in-house pipeline and included: AMG, hippocampus, caudate, putamen, and globus pallidus. Left and right ROIs were averaged.

Groups were compared on ROI volumes and brain-behavior correlations were conducted to examine the relationship between frustration exhibited during the task and ROI volumes. All analyses included age and Total Cerebral Volume (TCV) as covariates. Findings related to behavioral task performance and neuroimaging measures are not reported due to space limitations.

**Results:** Behavioral results showed that across all participants, the task elicited frustration from baseline to the frustrative block, $t(101) = -10.96$, $p \leq .001$; however, controlling for age, groups did not differ in change in frustration, $F(2,98) = .219$, $p = .804$

Controlling for age, groups differed in TCV, $F(2,76) = 3.49$, $p = .035$, such that the TDs had greater TCV compared to the Comorbid group ($p = .05$). Results of a MANCOVA examining all cortical ROI volumes revealed a significant multivariate main effect of Group, $\lambda = .800$, $F(8,148) = 2.19$, $p = .032$, due to reduced ACC volume in ADHD vs. TDC ($p = .003$) and reduced dlPFC volume in Comorbid vs. TDC ($p = .093$). For subcortical measures, the multivariate effect was not significant, $\lambda = .833$, $F(10,146) = 1.40$, $p = .186$; therefore, univariate tests were not examined.

Across all groups, partial correlations showed a significant negative association of dlPFC and mPFC volumes (respectively) with frustration at Baseline ($r = -.245$, $p = .028$; $r = -.327$, $p = .003$), after the frustrative block ($r = -.246$, $p = .028$; $r = -.276$, $p = .013$), and in change in frustration across the task ($r = -.240$, $p = .032$; $r = -.255$, $p = .023$). Further, partial correlations showed a significant positive relationship between baseline frustration and caudate volume ($r = .234$, $p = .037$).

**Conclusions:** Compared to TDCs, children with ADHD showed reduced volumes in prefrontal regions including the ACC and dlPFC. Moreover, on a novel frustration GNG task, reduced dlPFC and mPFC volumes and greater caudate volume were negatively associated with self-reported frustration suggesting the importance of neural circuitry involved in reward and cognitive control in frustration tolerance.

**Keywords:** Attention Deficit Hyperactivity Disorder, Frustration Tolerance, Emotion Regulation, Affective Neuroscience, Irritability

**Disclosure:** Nothing to disclose.

## T19. Differences in Brain Structure and Functioning in Children With the FTO Obesity Risk Allele

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**Background:** The prevalence of obesity (defined as body mass index (BMI) ≥ 95th percentile) in the US is estimated at 39% in adults and 18% in youth, highlighting the need to identify risk factors (Hales et al. 2017). In recent years, polymorphisms in the fat mass and obesity-associated gene (FTO) have been associated with higher BMI and risk for obesity (Frayling et al. 2007). In the FTO gene, (intronic single nucleotide polymorphism (SNP) rs1421085), CC and CT genotypes are considered at-risk genotypes compared to the TT genotype (Wang et al. 2013; Hudek et al. 2018; Albuquerque, Nóbrega, and Manco 2013). Whereas the mechanism underlying the association between FTO gene and obesity is not fully understood, individuals carrying risk alleles show higher food intake, regardless of BMI (Cecil et al. 2008; Wardle et al. 2008). As FTO is highly expressed throughout the brain, a growing body of literature has started to examine brain function and structure across FTO genotypes (McTaggart et al. 2011). Thus far, studies find volumetric reductions (Melka et al. 2013), differences in activation in response to food stimuli (Heni et al. 2014; Wiemerslage et al. 2016; Melhorn et al. 2018) and differences in connectivity in the default mode and salience networks (Olivo et al. 2016). However, studies have examined FTO genotype differences mostly in adult populations. Given that weight status has been shown to impact brain functioning (Doucet et al. 2018), the mechanisms leading to weight gain are better examined in children at risk for obesity, rather than in adults who are already overweight. The one existing child study supports a potential mechanistic role of brain function in the relation between FTO and weight gain, documenting stronger responses to food stimuli in regions involved in reward processing in risk allele carriers (Rapuano et al. 2017). However, the study sample had significant differences in BMI across FTO genotypes. We thus examined associations between FTO genotypes and brain structure and function in a sample of children with comparable BMIs.

**Methods:** Saliva was collected and DNA was extracted using DNA Genotek™ kit. Children were genotyped for the C/T alleles at rs1421085 by the pyrosequencing (PSQ96 Biotage, LLC. Westborough, MA). PCR reactions consisted of 6 pmol of each of the appropriate forward and reverse primer, 0.75 U GoTaq, 1xGoTaq buffer, 0.2 mM dNTP's and 50 ng of genomic DNA in a 30 μλ reaction volume for 35 cycles at an annealing temperature of 50°C. Genotype brain differences were examined in 93 children (15 with CC genotype, 31 with CT genotype, and 47 with TT genotype) ages 6-11 (mean age = 9.12 ± 1.17 years). Grey matter (GM) morphology, white matter (WM) fiber density, and resting-state functional connectivity (rsfMRI), were assessed using deformation-based GM morphometry, fixel-based morphometry (Raffelt et al. 2017), and seed-based analyses, respectively.

**Results:** Age [$F(2,90) = 0.38$, $p = 0.69$], sex [$\chi2(2) = 0.37$, $p = 0.83$], and BMI [$F(2,90) = 0.47$, $p = 0.63$] distributions did not vary across genotypes. GM morphology analyses demonstrated that C allele carriers showed significant GM volume expansions bilaterally in the cerebellum and temporal gyrus and in the right occipital cortex (whole brain corrected $p < 0.05$; adjusted for sex, age at scan, and BMI). Given these findings, we examined cerebral WM structural and functional connectivity. Fixel-based morphometry analyses showed a significant relationship between C alleles and increased WM fiber density in the inferior cerebellar peduncle tract (FWE corrected $p < 0.05$, adjusted for the aforementioned covariates). Finally, cerebellum seed-based rsfMRI analyses showed that C alleles predicted increased positive connectivity between the left cerebellum (lobule VI) and left superior frontal

gyrus and right thalamus as well as increased negative connectivity between the cerebellum (Crus II) and the temporal fusiform cortex and lateral occipital cortex (thresholded at voxel level $p < 0.001$ (uncorrected) and at cluster level $p < 0.05$ (FDR corrected); adjusted for the aforementioned covariates).

**Conclusions:** In line with prior research, the present study suggests that the FTO gene may impact brain structure and functioning. We document that FTO genotype differences occur independently of BMI and can be detected during childhood. Our multimodal analyses suggest a prominent role for the cerebellum in FTO genotype differences. Animal and human studies document that the FTO gene is widely expressed in the cerebellum (Fredriksson et al. 2008) and a prior adult study found genotype by reward sensitivity interactions such that for homozygote risk allele carriers, higher punishment sensitivity was related to increased rsfMRI cerebellar activity (Olivo et al. 2016). Given the cerebellum's role in reward based-learning (Thoma et al. 2008), and reports of increased cerebellar activity as a function of BMI (Park, Seo, and Park 2016), our results lend premilitary support to the hypothesis that FTO risk allele carriers may present atypical food-related learning and sensitivity, even prior to developing obesity and other adiposity-related conditions. However, further study is required to better elucidate the effects of the FTO genotypes on brain development, food intake, and obesity susceptibility.

**Keywords:** Obesity, Magnetic Resonance Imaging, FTO, Children

**Disclosure:** Nothing to disclose.

## T20. Developmental Origins of Adult Prefrontal Cortical Parvalbumin Interneuron Functional Dysconnectivity

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**Background:** Abnormalities in prefrontal cortical parvalbumin-expressing (PFC PV) interneurons are believed to contribute to cognitive and affective deficits in schizophrenia (SCZ), as well as other neurodevelopmental psychiatric disorders. However, little is known about whether developmental alterations in PV inhibitory interneuron maturation and integration into cortical circuitry could be contributing to disease onset. We have recently shown that mice exposed to an early environmental risk factor for SCZ—prenatal maternal immune activation (MIA)—show decreased functional inhibitory connectivity between PFC PV interneurons and pyramidal cells in adulthood, and that these physiological changes result in impairments in cognitive flexibility and anxiety. Therefore, we decided to utilize this model to investigate changes in PFC PV interneuron function during development that may precede and precipitate these long-term functional and behavioral alterations observed in the adult.

**Methods:** We used slice electrophysiology to record from PFC PV interneurons from MIA and control offspring at different time points during development and in adulthood. We then utilized a viral and genetic approach to express the pharmacogenetic receptor, hM4D, in developing PV interneurons and administered the agonist for hM4D, clozapine-N-oxide (CNO), during the postnatal window (P14-P50) to mimic the physiological changes in excitability we observed in MIA offspring during development. Twenty-four hours following the end of this transient pharmacogenetically-induced reduction in excitability, we measured the strength of GABAergic connectivity in the PFC by recording spontaneous inhibitory post-synaptic currents.

**Results:** We discovered that PFC PV interneurons in MIA offspring show decreased intrinsic excitability transiently during early development, corresponding to a window during which extensive pruning of synaptic connections occurs. This change in excitability appears due to an increase in an inward conductance active around resting membrane potential and at more hyperpolarized potentials in PFC PV interneurons. Our pharmacogenetic studies reveal that transiently reducing the excitability of PFC PV interneurons early in development is sufficient to reduce the strength of functional GABAergic connectivity between these interneurons and pyramidal cells 24 h following normalization of PV interneuron excitability. Persistent effects on GABAergic connectivity in adulthood are also detected. Ongoing studies are examining the effects of this developmental manipulation on PFC PV interneuron-dependent behaviors such as attentional set-shifting and anxiety.

**Conclusions:** Our results indicate a sensitive developmental period during which alterations in PFC PV interneuron excitability are able to affect the strength of GABAergic functional connectivity, even after excitability has returned to normal. This may have consequences for establishing the strength of prefrontal GABAergic connectivity in adulthood as well as for prefrontal PV-dependent behaviors. From a therapeutic perspective, this may indicate a developmental period during which the brain would be particularly susceptible to interventions that engage the prefrontal cortex and thereby naturally enhance activity.

**Keywords:** Parvalbumin Interneurons, Brain Development, Prefrontal Cortex, Excitability

**Disclosure:** Nothing to disclose.

## T21. Neural Coding of Explore-Exploit Decisions in Macaque Prefrontal Cortex

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**Background:** The explore-exploit dilemma describes agents' decisions to forego immediate rewards and explore an unknown option, to learn if it is better than something they already experienced. Previous studies find that cortical regions encode exploratory choices when primates deviate from specified decision policies. However, without explicit task constraints it is difficult to tell if off policy-choices reflect exploration, decision noise, or poor learning. Novelty seeking is an evolved solution to the explore-exploit dilemma and of interest because it is computationally tractable.

**Methods:** In the present study we combined high channel count single-unit recordings (768 electrodes; > 3000 neurons) from macaque prefrontal cortex (Area 9/46) with computational modeling of two monkeys' behavior on a multi-arm bandit task. The monkeys learned to choose between three, probabilistically rewarded images. Periodically one of the choices was replaced with a novel image the monkey had not yet associated with reward. This induced an explore-exploit tradeoff, forcing the monkeys to either explore the novel option or exploit their existing knowledge about the two remaining familiar options. We used a Partially Observable Markov Decision Process (POMDP) model to quantify the value of choosing each option based on the likelihood that choice would be rewarded on the current trial (immediate expected value) the overall richness of the reward environment (future expected value), and the relative difference in the total number of future rewards to be gained by choosing to explore or exploit novel versus familiar options (exploration bonus).

**Results:** Prefrontal neurons encoded each of these value computations, however, there were key differences in when these value signals were encoded. We observed both tonic encoding of the immediate and future expected value of choices during the inter-trial interval, as well as phasic encoding of these values at the

time of choice. Whereas, the exploration bonus tied to novelty seeking was only encoded after the monkeys chose to explore or exploit particular options. Prefrontal neurons also encoded the identity of the chosen stimulus and choice outcomes. This is important because the immediate expected value, stimulus identity, and outcome of choices defines the state and the state transition in the POMDP algorithm we used to derive choice values. Interestingly, choice location which was task irrelevant but important for action selection, was also strongly encoded.

**Conclusions:** Overall, these results suggest that prefrontal cortex is important in resolving uncertainty about the value of unexplored, novel options to efficiently manage the explore-exploit dilemma.

**Keywords:** Reinforcement Learning, Prefrontal Cortex, Explore-Exploit Dilemma, Probabilistic Reward Learning, Computational Models Of Decision-Making

**Disclosure:** Nothing to disclose.

### T22. Contributions of Parallel Nigrostriatal Dopamine Circuits to Reward Learning and Habit Formation

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**Background:** Habit formation, a process by which behavior becomes fluid and automatic, is an essential survival strategy in a complex world, freeing cognitive resources for other purposes. The process of habit formation depends on the striatum and inputs to the striatum from the substantia nigra pars compacta (SNc). Our recent work has shown that dopamine neurons in SNc are divisible by their efferent projections to the dorsomedial striatum (DMS) or dorsolateral striatum (DLS), striatal subregions that play distinct roles in operant learning: the DMS mediates early goal-directed action-outcome learning, while the DLS mediates later habit formation. Now, we are asking whether changes in nigrostriatal dopamine circuits' structure and function are altered by habit formation and how these different classes of efferent-defined dopamine neurons differentially contribute to reward learning and the transition to habit.

**Methods:** To address the question of how nigrostriatal dopamine circuits change over the course of habit formation, we are using both optogenetic circuit mapping in ex vivo slices and in vivo fiber photometry recordings during behavior. We injected ChR2-YFP into either the DMS or DLS and red retrobeads into either the DMS or DLS. This preparation allows us to identify DMS-projecting or DLS-projecting dopamine neurons in ex vivo slices to target for our recordings. We can then stimulate ChR2-expressing axons with blue light and test the connectivity of the DMS and DLS onto these efferent-defined dopamine neuron populations. We are currently comparing the connectivity in mice that have been trained to form habits with cagemates that are similarly handled and food restructure, but untrained. For fiber photometry recordings, we injected DIO-GCaMP6s into the SNc of DAT-cre mice to express GCaMP6s in SNc dopamine neurons. Fiber optic implants were placed in both the DMS and DLS, allowing us to simultaneously measure the activity of dopaminergic axons in these two striatal subregions as mice trained on an operant task designed to elicit inflexible behavior. Since individual mice acquire habits are different rates, these experiments allow us to identify the dopaminergic circuit signatures of habit formation vs changes that are independent of habit formation. These preliminary experiments are ongoing, with statistical analyses forthcoming. Both sexes are being examined and we will test for sex-dependent effects in our analyses.

**Results:** In our preliminary studies, we have observed selective alterations of striatal inputs onto DLS-projecting dopamine neurons following habit training. The strength of DMS inputs onto DLS-projecting dopamine neurons increases, whereas the strength of DLS inputs onto these neurons decreases. In our fiber photometry recordings, we have observed that dopamine signals in the DLS preferentially evolve over the course of habit training, in particular showing a prolonged increase in activity in response to salient events, independent of reward outcome.

**Conclusions:** So far, our preliminary results suggest that plasticity of inputs onto DLS-projecting dopamine neurons plays a key role in habit formation. Future experiments will address the timeline and mechanisms by which this plasticity occurs. We also plan interventional optogenetic experiments to test whether the timecourse of habit acquisition and consolidation can be altered via temporally precise manipulations of the DLS-projecting dopaminergic system.

**Keywords:** Dopamine, Dorsal Striatum, Habit, Reward Learning, Optogenetics

**Disclosure:** Nothing to disclose.

### T23. Perimenstrual Exacerbation of Symptoms in Female Suicide Attempters With Current Ideation: Examining RDoC Mechanisms of Steroid Withdrawal in a Crossover Randomized Controlled Trial

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**Background:** The perimenstrual weeks of the menstrual cycle (i.e., weeks before and during menses, when ovarian steroids 17β-estradiol (E2) and progesterone (P4) fall rapidly), have been linked to suicide attempts and deaths. Our recently-completed crossover double-blind trial of perimenstrual E2/P4 stabilization in female outpatients with a history of suicide attempt (and current ideation) provides evidence that perimenstrual withdrawal from E2 and P4 causes increases in suicidality, which can be prevented using exogenous steroid stabilization. Now, candidate mechanisms require exploration. In preclinical models, ovarian steroid withdrawal provokes a variety of behavioral phenotypes (depression, impulsivity) related to affective and suicide risk. Using data collected during the trial referenced above, the present work examines candidate RDoC mediators of the observed therapeutic effect. We hypothesized that prevention of perimenstrual steroid withdrawal would prevent deterioration of RDoC systems known to influence acute suicidality, including loss, potential threat, reward processing, behavioral disinhibition, social affiliation and attachment, and disturbed sleep.

**Methods:** Procedures were approved by the UNC Chapel Hill Ethics Board. In a crossover double-blind placebo-controlled trial, female participants ($n = 25$) with a history of suicide attempt and current ideation were recruited for a study on "the biology of depression and suicide" via social media. Participants completed two 14-day conditions in counterbalanced order: In the active condition, they were treated with 14 days (starting 7 days following positive urine LH test) of transdermal E2 (.1 mg/day) patches plus oral micronized P4 (200 mg) pills; in the other condition, they received identical placebos in the same perimenstrual timeframe. A washout cycle separated the conditions. Participants (ages 18-45) had normal BMI, no premenstrual dysphoric disorder (luteal phase symptom confinement), serious medical conditions, dysmenorrhea, pregnancy/breastfeeding, hormone use, or history of mania or psychosis.

Participants reported daily suicidality and indicators of loss ("I felt sad", "I felt hopeless"), potential threat ("I felt anxious", "I felt worried"), blunted reward processing ("I had little motivation or interest", "I did not enjoy things"), cognitive/behavioral disinhibition ("I did something impulsive I regret", "I acted without thinking"), impaired social affiliation and attachment ("I felt I did not belong with others", "I felt I was a burden to others", number of social interactions), and poor sleep ("I had trouble falling or staying asleep", number of hours of sleep reported). Composites were calculated as the daily mean of within-person z-scores. Multilevel models were used to test hypotheses. Physical symptoms and medications were covaried.

**Results:** All participants demonstrated a 30% perimenstrual exacerbation of at least one emotional symptom. suggesting high risk of cyclical exacerbation in this population. Serum measures across each condition confirmed that the manipulation extended the mid-luteal phase hormone profile, preventing E2/P4 withdrawal relative to perimenstrual steroid withdrawal under placebo. Blinding was successful, with no condition differences in condition beliefs ($p = .58$).

Consistent with previously-reported beneficial effects on suicidality in this trial, we observed condition X phase interactions predicting composite outcomes related to loss (Estimate = -.74, SE = .25, $t(905) = -2.96$, $p = .03$), blunted reward processing (Estimate = -.60, SE = .24, $t(902) = -2.50$, $p = .012$), cognitive/behavioral disinhibition (Estimate = -.50, SE = .22, $t(907) = -2.27$, $p = .023$), impaired social affiliation (Estimate = -.48, SE = .11, $t(905) = -4.36$, $p < .0001$), and poor sleep (Estimate = -.84, SE = .20, $t(905) = -4.20$, $p < .0001$). During placebo, these outcomes worsened one to one-and-a-half person-standard-deviations from the midluteal to the perimenstrual week but showed no increase during stabilization. Further, there were significant condition X phase effects on these outcomes during the medication withdrawal week; delayed withdrawal from active E2/P4 caused a delayed increase in symptoms relative to midluteal baseline (not observed during placebo withdrawal). There were no significant condition or condition X phase effects on potential threat (Estimate = -.07, SE = .26, $t(901) = -.26$, $p = .79$).

**Conclusions:** In addition to exacerbating daily suicidality, ovarian steroid withdrawal (both natural perimenstrual and experimental withdrawal) provoked heightened sadness, hopelessness, anhedonia, impulsivity, social disconnection, and trouble sleeping in a sample of female suicide attempters with current suicidal ideation. Anxiety was not affected and may not be a key mechanism. Studies should probe whether disturbed sleep may represent a mechanism between steroid withdrawal and psychiatric symptoms.

Perhaps due to shared risk factors, chronic suicidality in females seems to correlate with psychiatric sensitivity to perimenstrual steroid withdrawal. This withdrawal mechanism stands in contrast to those observed in premenstrual dysphoric disorder, where symptoms are triggered by post-ovulatory neurosteroid changes rather than perimenstrual steroid changes. Serum from the present study is currently being assayed to examine possible molecular mechanisms, such as GABAergic neurosteroid withdrawal.

**Keywords:** Suicide, Gonadal Hormones, Sex Hormones, Women's Mental Health, Research Domain Criteria (RDoC)

**Disclosure:** Nothing to disclose.

## T24. Social Cognition Remediation for Bipolar Disorder - A Path to Better Clinical Outcomes

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**Background:** Bipolar disorder (BD) is associated with poor emotion recognition (Samamé, 2013). Misinterpreting others' emotions may exacerbate disordered mood; for people with BD, who tend to have a negative emotion bias, noticing and focusing on negative emotions in others may maintain depressed mood (Leppanen, 2006). This effect on mood could be due, in part, to the social functioning deficits associated with misinterpreting others' emotions (Fulford et al, 2014; Michalak et al., 2006).

This emotion recognition deficit – and associated consequences – appear to be specific to faces; recently completed work by our group suggests that people with BD perform as well as others when asked to interpret emotional body language (Lee & Van Meter, 2018). Remediating the face emotion recognition deficit could (1) reduce depressed mood in people with BD, (2) improve social functioning, and (3) increase treatment engagement.

Previous research has indicated that a computer-based cognitive bias modification intervention designed to modify emotion perception biases may positively influence mood and behavior in depressed and aggressive individuals (Penton-Voak et al., 2012; 2013). Our goals are to determine whether this intervention can shift emotion processing biases in people with BD and demonstrate clinical benefits (lower depressed mood, improved social function, greater treatment engagement).

**Methods:** Young adults (16-25) with BD were recruited for a three weekly sessions of Interpretation Bias Training (IBT), a computer-based intervention that trains sad/happy judgments of ambiguous face emotions on a linear morph continuum of faces between sad and happy (Penton-Voak et al., 2012). Active IBT targets negative emotion bias by training towards more happy judgments of ambiguous faces on the sad/happy continuum, relative to a person's native bias. Feedback during sham IBT is consistent with a person's native bias. Exclusion criteria were minimal. Participants were interviewed to assess eligibility and completed mood and social functioning self-reports. Participants were randomized to the active or sham condition. Two months after intervention sessions, emotion recognition, mood (General Behavior Inventory, (Depue, 1981), and social functioning (Perceived Social Support – Family and Friends (Procidano & Heller, 1983) were evaluated. Changes in treatment (start/stop medication or therapy) were also assessed to account for engagement.

A small subset of participants ($n = 7$) in the active condition underwent neuroimaging pre- and post-intervention to assess within-person changes in neural activation during a separate emotion processing task (participants viewed faces exhibiting different emotions and were asked to identify the gender of the face).

**Results:** Forty-three participants (average age 21.89, $SD = 2.4$, 70% female) completed at least one intervention session, 79% completed all four sessions. The most common subtype was BD I ($n = 29$) followed by BD II ($n = 9$). At baseline, 59% were in psychotherapy, 60% were currently taking medication for their BD. The active group had higher depression scores than the sham group ($t = 2.30$, $p = .027$), other measures were equivalent.

The negative emotion bias shifted more in the active than in the sham group (Cohen's $d = 1.49$, $p < .001$), indicating the training was effective.

In a linear mixed model, with random intercepts for participant and a random slope for the measure of negative emotion bias, fixed effects for group ($p = .027$) and group-by-time ($p = .031$) were significant; the intervention group had a larger decrease in depressed mood from baseline to two-month follow-up. ($p = .027$). Related, the intervention group had a larger increase in perceived social support from family members ($p = .011$). There were no main effects for group, time, or their interaction on mania scores or treatment status (initiating medication or participating in psychotherapy).

Among those participants who underwent neuroimaging pre- and post-intervention, at baseline there was significant activation

in the dorsal medial prefrontal cortex (DMPFC) when observing the emotional faces (happy, sad). Following the intervention, there was no significant activation in this area.

**Conclusions:** A computer-based cognitive bias intervention shifted emotion perception in people with BD, which may have contributed to decreased depression scores and the perception of greater familial support, consistent with the "virtuous cycle" theory that seeing others' emotions more positively can lead to enhanced interactions and improved mood (Penton-Voak, 2012; Penton-Voak et al., 2013). A reduction in DMPFC response after intervention provides preliminary support for it as a neural treatment target and is consistent with its role in emotion regulation, identification, and learning (Bakker et al., 2015).

These results converge with prior work demonstrating IBT may reduce depressed mood in healthy individuals (Penton-Voak et al., 2012). On average, people with BD spend more time depressed than manic, and depressed mood tends to be more difficult to treat (Baldessarini et al., 2010; Van Meter et al., 2013). The results of this study suggest that cognitive remediation could augment treatment with medication and/or therapy targeting BD depression through emotion processing and social pathways. Importantly, this computer-based intervention can be more easily disseminated, at lower cost, than traditional interventions, creating an opportunity to benefit under-served populations.

**Keywords:** Bipolar Disorder, Social Cognition, Non-Pharmacological Interventions, Mobile Health

**Disclosure:** Nothing to disclose.

### T25. Using Resting State Intrinsic Network Connectivity to Identify Suicide Risk in Mood Disorders

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**Background:** Little is known about the neural substrates of suicide risk in mood disorders. Improving the identification of biomarkers of suicide risk in mood disorders could lead to more targeted treatments to reduce risk. The aim of this study was to use resting-state intrinsic network connectivity to identify individuals at risk for suicide, as indicated by a history of suicide-related behavior (SB).

**Methods:** A cross-sectional study was conducted at two urban communities with medical centers. Resting-state functional connectivity was examined within intrinsic networks, including the cognitive control network (CCN), a system involving fronto-parietal and dorsal attention networks that is critical for problem-solving and executive functioning; the salience and emotional network (SEN), which is active in response to stimuli relevant to current goals, including emotional stimuli, and involves limbic and ventral attention networks; and the default mode network (DMN), which is active during self-focused thought and when not engaged with external stimuli. Two fMRI scans were conducted approximately two months apart to examine stability and reliability of group differences over time. Participants (Mean age = 21.88, SD = 2.70; 67% female) were 112 individuals with a mood disorder with no history of suicide-related behavior (NSB), 18 young adults with a mood disorder who had a history of SB (as indicated by endorsing a past suicide attempt), and 82 healthy comparison participants (HC). Strength of resting-state connectivity of intrinsic networks was compared between SB, NSB, and HC groups.

**Results:** Several regions ($k > 57$, $p < .005$) were identified in the three networks in connectivity to fronto-parietal regions, including right middle and inferior frontal gyrus and inferior parietal lobule, that were significantly different in SB relative to NSB and HC groups for both within-network connectivity (in the CCN) and cross-network connectivity (DMN-CCN and DMN-SEN). Furthermore, deficits in connectivity (exhibited by the SB group) between the right middle frontal gyrus and the CCN were associated with poorer inhibitory control on a behavioral go/no-go task, and deficits in connectivity between the right middle frontal gyrus and DMN were associated with higher levels of self-reported rumination. Intrinsic network connectivity effects were stable over time and identified group membership with good accuracy, sensitivity, and specificity.

**Conclusions:** These results suggest that individuals with a history of SB may show distinct patterns of intrinsic network connectivity, even when compared to those with mood disorders and no history of SB. These deficits may underlie candidate behavioral risk factors for suicidal ideation and suicidal behavior, including rumination and inhibitory control deficits. Resting-state fMRI may serve as a promising tool for identifying subtypes of patients with mood disorders who are at risk for suicidal behavior.

**Keywords:** Suicide, Resting-State fMRI, Depression, Intrinsic Connectivity, Cognitive Control Network

**Disclosure:** NIMH, Grant, BBRF, Grant

### T26. Frontoinsular Network Markers of Current and Future Adolescent Mood Health

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**Background:** Adolescence is a developmental period in which depression and related mood syndromes often emerge. However, few objective markers exist to guide diagnosis or predict mood health in youth. The present study addressed this gap using task-based neuroimaging to identify frontoinsular biomarkers of current and prospective mood health in adolescents.

**Methods:** Adolescents ($n = 42$, ages 13-19, 72% female) reporting varying levels of depressive symptom severity completed a baseline session that included neuroimaging during an emotional executive functioning task. Next, teens ($n = 28$) completed a two-week follow-up consisting of a daily diary report of negative affect and a final report of depressive symptom severity (day 15). Analyses tested associations between task-related functional connectivity in frontoinsular networks involved in attention regulation, and baseline or prospective measures of mood health. Sex was considered as a biological variable of interest.

**Results:** Frontoinsular task response was significantly associated with poorer mood health: higher current depression severity (partial eta squared = 0.12), increases in future depressive symptom severity (partial eta squared = 0.23), and more intense and labile negative affect in daily life (partial eta squared = 0.22-0.30). In particular, task-related hypoconnectivity between insula and lateral prefrontal regions of the frontoparietal network, and hyperconnectivity between insula and midline or temporal regions of the default network, were associated with poorer mood health. Prospective associations between frontoinsular functioning and mood health were significant when controlling for baseline symptoms, indicating that biomarkers complement other sources of risk information.

**Conclusions:** Findings for frontoinsular imbalances as a biomarker of current and prospective mood health in adolescence suggest that network functioning may hold promise as a

translational tool to guide risk prediction and inform preventive treatments.

**Keywords:** Adolescent Depression, Task-Based Functional Connectivity, Working Memory, Emotion Regulation, Clinical Prediction

**Disclosure:** Nothing to disclose.

### T27. Depression Screening Rates and Symptom Severity by Alcohol Use Among Primary Care Adult Patients

Abstract not included.

### T28. Electroconvulsive Therapy Modulates Loudness Dependence of Auditory Evoked Potential: A Meg Study

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**Background:** Electroconvulsive therapy is the most effective treatment for major depressive disorder (MDD). The understanding of the neurobiological mechanism underlying ECT efficacy remains incomplete. Previous studies have reported ECT-induced changes in multiple neurotransmitter systems, including the serotonin (5-hydroxy-tryptamine, 5-HT) system. Serotonin has been shown to modulate various physiological processes, such as thermoregulation, and behavioral functions, such as the sleep-wake cycle, aggression, mood and anxiety, sexual behavior, and learning. Thus, pathological changes of central serotonergic neurotransmission are associated with an array of psychiatric conditions, including MDD. An indicator of serotonergic activity is the loudness dependence of auditory evoked potential (LDAEP). LDAEP refers to the change in amplitude of evoked N100/P200 components in response to different auditory stimulus intensities; it is thought to be a measure of the modulation of activity in the auditory cortex by serotonergic neurons originating in the dorsal raphé. Growing evidence suggests that LDAEP may be biological markers for predicting response to antidepressants. For example, studies showed that patients with MDD exhibiting high LDAEP prior to treatment are more likely to respond favorably to antidepressants compared to those exhibiting low LDAEP. This study seeks to assess the effect of ECT on serotonergic neurotransmission using the LDAEP paradigm and magnetoencephalography. Previous work has shown that 1) MDD is associated with decreased central serotonin levels, 2) ECT decreases 5HT-1A receptor binding, and 3) an inverse relationship between LDAEP and central serotonin levels. Thus, we hypothesized that ECT would decrease LDAEP through increase in central serotonergic neurotransmission.

**Methods:** This study was approved by the Institutional Review Board of University of New Mexico. The 24-item Hamilton Depression Rating Scale (HAMD-24) was used to assess severity of depressive symptoms. Nine patients with MDD (6 female, age 68.1 + /- 10.7, baseline HAMD-24 = 37.2 + /- 12.8) eligible for ECT participated in this study. Patients received a standard course of ultrabrief pulse width right unilateral ECT. Structural MRI and magnetoencephalography (MEG) were acquired before and after the ECT course. MEG was recorded using the Elektra Neuromag VectorView 306 system, equipped with 102 magnetometers and 204 planar gradiometers, in addition to electrocardiography and electrooculography recordings. For the auditory evoked potential task, subjects listened to a series of tones at five intensity levels (55, 65, 75, 85, 95 dB, 2 kHz frequency for 50 ms; randomized blocks of 22 tones for a total of 110 trials per intensity) through biauricular earbuds. Brainstorm 3 was used for MEG data processing and visualization. Individual head models were reconstructed from structural MRI using Freesurfer, and coregistered to the scalp fiducials. The MEG signal was bandpass filtered from 1 Hz to 100 Hz and notch filtered at 60 Hz to remove powerline interference. Faulty channels were manually removed. Heartbeat and eyeblink artifacts were removed using signal-space projection method. Independent component analysis was used to remove additional non-brain artifact. The data was epoched to -100 to 500 ms relative to stimulus delivery time, and z-score normalized to 100 ms of prestimulus baseline. Source reconstruction was done using the linearly constrained minimum variance (LCMV) beamformer method. The trial-averaged, source-level, event-related field (ERF) was extracted from the bilateral primary auditory cortices (PAC) of each subject. The change in ERF amplitude between the N100 and P200 peaks was extracted and plotted as a function of tone intensity, the slope of the linear fit defines the loudness dependence of the auditory evoked potential. Finally, the LDAEP was compared before and after ECT treatment using paired t-test.

**Results:** Seven of the nine patients responded to ECT (six remitters, post treatment course HAMD-24 = 9.1 + /- 7.6). The mean pre-ECT LDAEP was 0.4125 per dB; the post-ECT LDAEP was 0.7527 per dB, significantly increased from baseline ($p = 0.013$). The association between the change in LDAEP and HAMD-24 was negative, albeit not statistically significant.

**Conclusions:** Contrary to our hypothesis, the results indicate a greater dependence of auditory evoked potential on loudness levels after the ECT treatment course. This leads us to an alternative hypothesis, that the antidepressant effects of ECT are in part mediated by increased dopamine neurotransmission, which has been shown in a previous study to be positively associated with increased LDAEP. Further studies are required on the relationships between ECT, LDAEP, dopaminergic and serotonergic neurotransmission.

**Keywords:** Electroconvulsive Therapy, Magnetoencephalography, Serotonergic System, Dopaminergic System

**Disclosure:** Nothing to disclose.

### T29. An Integrative Network Analysis Identifies Altered Immune Signaling Pathways in Treatment Response to Antidepressants

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**Background:** Patient response to antidepressant varies greatly, likely contributed by multiple factors, including mutations, altered signaling and metabolic pathways. This project aimed to integrate DNA, microRNA and mRNA data to discover molecular pathways associated with treatment response to antidepressants using an integrated network analysis approach.

**Methods:** Biological samples were obtained from three antidepressant clinical trials (11918 A, 11984 A, 13267 A). The cohort included patients treated with duloxetine, and data comprised DNA samples from 186 subjects, microRNA and mRNA samples from 124 subjects. The molecular data was integrated using mirDIP, IID and pathDIP, annotated resources for microRNA: gene predictions, protein:protein physical interactions, and comprehensive pathway enrichment analysis (http://ophid.utoronto.ca/mirDIP, …/iid, …/pathDIP).

**Results:** Analysis of individual data sources yielded no significant results; however, network-based analysis across

microRNA, RNA and DNA samples identified 1,142 significantly enriched pathways. Immune signaling pathways emerged as the most significant pathways both individually and upon systematic domain specific classification. The top immune signaling pathways were then successfully validated for differential expression among predicted responders to duloxetine.

**Conclusions:** The results confirmed currently implicated pathways and pointed towards novel pathways for further exploration. In particular, specific pathways such as TRAF6 emerged as key players in antidepressant treatment response to duloxetine. Within the validated immune signaling pathways could exist proteins that serve as unique molecular targets to decipher the biology of antidepressant treatment response. The integrative network analysis approach demonstrates that variations of a small effect size at the molecular level can aggregate within pathways that could be meaningfully explored and targeted in antidepressant response.

**Keywords:** Biomarker, Network-Analysis, Antidepressant Response

**Disclosure:** Nothing to disclose.

### T30. Opioid Receptor Antagonism Attenuates Antidepressant Effects of Ketamine

Abstract not included.

### T31. (2 R,6 R)-Hydroxynorketamine Exerts mGluR2-Dependent Antidepressant Actions

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**Background:** Numerous clinical studies have provided strong evidence that ketamine rapidly (within hours of administration) improves depressive symptoms in treatment-resistant depressed patients following a single infusion. In preclinical animal tests, ketamine's metabolite (2 R,6 R)-hydroxynorketamine (HNK) shows promise as a rapid-acting antidepressant candidate drug that lacks ketamine's side effects, including dissociation and abuse liability. Metabotropic glutamate receptor 2/3 (mGluR2/3) antagonists exert rapid and sustained antidepressant actions in rodents and have been suggested to possibly share similar mechanisms of action with ketamine.

**Methods:** Using behavioral, pharmacological and genetic approaches, complemented by in vivo quantitative electroencephalographic (EEG) measurements, we examined the role of mGluR2 and mGluR3 receptors in the antidepressant behavioral actions of (2 R,6 R)-HNK. All experimental procedures were approved by the University of Maryland, Baltimore Animal Care and Use Committee and were conducted in full accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

**Results:** Ketamine and (2 R,6 R)-HNK prevented mGluR2/3 agonist-induced hyperthermia, which was not observed with other N-methyl-D-aspartate receptor (NMDAR) antagonists or a chemical variant of ketamine that hinders its metabolism to (2 R,6 R)-HNK. Combined sub-threshold doses of the mGluR2/3 antagonist LY341495 and (2 R,6 R)-HNK exerted synergistic antidepressant behavioral actions. Furthermore, we show that the antidepressant actions of (2 R,6 R)-HNK are absent in mice lacking mGluR2 (Grm2-/-), but not mGluR3 (Grm3-/-), and were also prevented by pre-treatment with an mGluR2/3 agonist (LY379268). (2 R,6 R)-HNK-induced increases in gamma EEG power were also absent in Grm2-/-, but not Grm3-/-, mice and in mice pre-treated with LY379268.

**Conclusions:** These findings indicate convergent mechanisms underlying the antidepressant actions of (2 R,6 R)-HNK and mGluR2 antagonists. Our data also support a role of enhanced high-frequency EEG activity as a marker of target engagement and synaptic activity, which underlie rapid antidepressant efficacy. Overall, these data support the use of drugs with mGluR2 antagonist activity in experimental therapeutic clinical trials either alone or in combination with ketamine or (2 R,6 R)-HNK, for treatment-resistant depression.

**Keywords:** Ketamine, Hydroxynorketamine, Depression, Antidepressant, mGlu 2/3 Receptor

**Disclosure:** Co-authors in patent applications related to the pharmacology and use of (2 S,6 S)- and (2 R,6 R)-hydroxynorketamine in the treatment of depression, anxiety, anhedonia, suicidal ideation and post-traumatic stress disorder, Patent

### T32. Ketamine Downregulates the Expression of Heat Shock Protein 70 in Patients With Treatment-Resistant Major Depressive Disorder

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**Background:** Heat shock proteins (HSPs) are ubiquitously expressed intracellular proteins that function as chaperones and assist in the synthesis and folding of proteins in the absence of stress. However, both animal models and human data suggest that HSPs are robustly upregulated in response to physical, cellular, and psychological stress. While intracellular HSP expression aids in the repair and stabilization of proteins during stress, the passive (during necrosis or apoptosis) or active release of these proteins into the extracellular matrix leads to increased production of pro-inflammatory cytokines. Of the known HSPs that mediate inflammatory response to stress, HSP70 has been implicated in psychiatric disorders, including major depressive disorder (MDD); indeed, multiple studies have shown increased HSP70 serum levels in patients at risk for or diagnosed with MDD. Other studies have found that increased HSP70 levels correlate strongly with symptom severity in patients with chronic negative affect. This study sought to investigate whether the glutamatergic regulator ketamine affects the expression of HSP70 in individuals with treatment-resistant MDD.

**Methods:** This double-blind, placebo-controlled, randomized, crossover study included 38 medication-free males and females with MDD and 23 healthy controls (HCs) ages between 18-65 years old. Ketamine and placebo infusions were administered two weeks apart; the study was designed to test the antidepressant efficacy of ketamine. Initial leads for the HSP70 were obtained from an initial pilot proteomic study of looking at MDD patient that are extreme ketamine responders, non-responders and the HCs individuals. Targeted plasma concentrations of HSP70 were measured in both arms of the study at baseline (60 minutes pre-infusion) as well as at three post-infusion timepoints: 230 minutes, Day 1, and Day 3 post- infusion. Age, sex, and body mass index were used as covariates.

**Results:** A marginal increase in HSP70 levels that nevertheless reached statistical significance was observed in MDD subjects compared to HCs; for both subject groups, baseline HSP70 levels did not correlate with severity of depressive symptoms (MDD p

= .30, HC p = .62). Results of a mixed-effect linear model indicated that across all subjects, HSP70 levels were significantly decreased in response to ketamine compared to placebo at Day 1 (p = .0008) and Day 3 (p = .004). Although the magnitude of the drug effect was larger in MDD participants than in HCs, this difference was not statistically significant (drug by group interaction, Day 1: p = .37, Day 3: p = .81). Thus, while ketamine decreased the severity of depressive symptoms, this change did not appear to be mediated by changes in HSP70 levels.

**Conclusions:** These preliminary findings suggest that ketamine affects HSP70 levels by decreasing stress and inflammation. This study is the first to show that ketamine impacts stress-regulating factors such as HSP70 in both MDD and HC subjects. Additional hypothesis-driven mechanistic studies designed to test the impact of subanesthetic ketamine on HSP70 expression are needed to further explore this initial finding.

**Keywords:** Major Depressive Disorder (MDD), Ketamine, Inflammation, Acute Stress, Heat Shock Protein 70

**Disclosure:** Nothing to disclose.

### T33. Developmental Origin of Sex Differences in Adult Mood and in Stress-Induced Transcriptional Coherence Across Mesocorticolimbic Circuitry

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**Background:** Women are approximately two times as likely to be diagnosed with major depressive disorder (MDD) compared to men. These sex differences in MDD might be driven by circulating gonadal hormone differences between men and women. Indeed, both female and male gonadal hormones influence mood in humans (e.g., premenstrual dysphoric disorder in women and low testosterone in men) and in animals. However, MDD prevalence remains higher in women across life stages and hormonal states, suggesting that other sex-related factors contribute to higher rates of MDD in women. We hypothesized that these sex differences could be driven by permanent organizational effects of gonadal hormones acting during sensitive developmental periods and/or due to effects of genetic sex.

**Methods:** Using the Four Core Genotypes (FCG) mice, we independently examined effects of genetic and developmental gonadal sex on baseline and chronic stress-induced anhedonia-/depressive-like behaviors (N = 15-20/group). Mice were gonadectomized in adulthood to eliminate circulating hormone effects and isolate the role of developmental hormones. We then performed RNA-sequencing in three mood-relevant brain regions [prefrontal cortex (PFC), basolateral amygdala (BLA), nucleus accumbens (NAc)] in non-stressed and stressed male and female mice ($p < 0.05$; fold change > 1.3). We next examined transcriptional coherence across brain regions in response to stress using the rank-rank hypergeometric overlap test (RRHO). We also used an integrative network approach [Weight gene co-expression analysis (WGCNA), the module differential connectivity (MDC) metric, and assessment of hub gene networks] to identify transcriptional modules and stress-specific hub genes that regulate stress susceptibility, with a focus on whether these differed by sex. All experimental protocols in animal studies were approved by the Institutional Animal Care and Use Committee and were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

**Results:** Under non-stress conditions, gonadal males exhibited lower anhedonia-/depressive-like behavior than gonadal females (novelty-suppressed feeding: $p < 0.01$; social conditioned place preference: $p < 10\text{-}5$). There were no genetic sex differences in behavior ($p > 0.3$ for both behavioral tests). Surprisingly, the gonadal sex difference was eliminated in chronically-stressed mice ($p > 0.2$ for both behavioral tests). Examination of the top biological pathways represented by the genes differentially-expressed between gonadal males and females under non-stress conditions were related to immune function ($p < 0.05$). We then probed the genes affected by chronic stress (nonstress vs. stress) and found very little overlap between males and females. However, in the NAc and PFC, the top biological pathways affected by stress were related to immune function in both males and females; the pathways in the BLA affected by stress were completely distinct in males and females. The RRHO analysis revealed sex-specific coherence patterns between brain regions in response to stress. In gonadal males, there was transcriptional coherence between the PFC and BLA in response to stress (-log10pvalue > 150); this pattern did not occur in females. Rather, chronic stress induced transcriptional coherence between the NAc and BLA in gonadal females (-log10pvalue > 150). WGCNA identified gene modules affected by stress, and the MDC metric showed that some of these modules were affected in opposite directions in males and females. Network analysis of the modules affected in opposite directions in males and females predicted potential upstream regulators of the sex-specific effects on gene expression patterns.

**Conclusions:** Since all mice had equivalent circulating hormone exposure in adulthood, these results suggest that sex differences in gonadal hormone exposure during sensitive periods of development program adult sex differences in mood. The sex differences in behavior might be driven by sex differences in immune-related genes. The cross-brain region coherence analysis suggests that stress induces transcriptional synchrony between the PFC and BLA in males, but synchrony between the BLA and NAc in females; this suggests that stress affects the mesocorticolimbic circuit differently in males and females. Further, the network analysis identified upstream regulator genes that might drive sex differences in stress susceptibility. Future studies will manipulate these predicted upstream regulators to determine their role in stress susceptibility.

**Keywords:** Sex Difference, Mesocorticolimbic, Depression-Like Behavior, RNA Sequencing

**Disclosure:** Nothing to disclose.

### T34. Mechanisms Underlying the Antidepressant-Like Behavioral Effects of Fluoxetine

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**Background:** Depression and anxiety disorders are a major cause of disability worldwide. Currently, selective serotonin reuptake inhibitors (SSRIs) are the most commonly prescribed medications for treating these disorders. However, only about half of patients respond to SSRI treatment, and even within this treatment-responsive population there is a delayed onset of therapeutic efficacy of several weeks. There is thus a considerable need for better and faster acting antidepressants. One way to develop novel antidepressants is to better understand how SSRIs work and then directly target the underlying mechanisms. While previous work has identified the hippocampus as a site of action for antidepressant drugs, the exact mechanisms by which the

hippocampus mediates the antidepressant effects of SSRIs remain unclear. Several lines of evidence now indicate that the endogenous opioid system may be involved in emotional regulation, and several components of the opioid system are co-expressed in the hippocampus, so it is possible that the antidepressant effects of SSRIs may be at least partly mediated by downstream interactions with the opioid system. Here, we explore this possibility by performing a gene analysis after inducing a model of anxiety/depression and antidepressant treatment, and then using that analysis to guide additional experiments in which we assess the antidepressant effects of SSRIs in mice that lack specific opioid system components.

**Methods:** To identify novel molecular targets that mediate SSRI treatment responses we induced a model of stress and antidepressant treatment. In this experiment, we treated wild-type mice chronically with corticosterone (35 μg/mL) for 8 weeks and administered fluoxetine (a commonly prescribed SSRI) during the last 28 days of corticosterone treatment. Antidepressant behavioral responses were assessed via novelty suppressed feeding (NSF) and forced swim tests that were performed at the end of the 8-week paradigm. Tissue was collected after the completion of all behavioral testing and RNA from the dentate gyrus was processed on an Illumina microarray. After performing the gene expression analysis, we identified proenkephalin (PENK), which encodes a precursor to the endogenous mu opioid receptor (MOR) ligand enkephalin, as a gene of interest. This was also independently confirmed by additional experiments in our laboratory using RNA sequencing. Therefore, in experiment 2, we assessed the role of MOR in mediating the antidepressant behavioral effects of fluoxetine. Wild-type mice or mice that were lacking MOR underwent the same stress and antidepressant treatment as in experiment 1, and were then tested in a battery of behavioral assays

**Results:** Overall as a group, fluoxetine-treated mice exhibited shorter latencies to feed in the NSF test and shorter durations of immobility in the forced swim test as compared with their vehicle-treated counterparts. However, there was some individual variability in the fluoxetine-treated group, with only ~70% of animals responding to treatment. When comparing gene expression profiles, we found genes that were significantly regulated in the dentate gyrus of responders relative to vehicle-treated controls, but not in non-responders. Notably, PENK was among the most upregulated genes, so we next assessed the efficacy of fluoxetine in MOR knockout (KO) mice. Relative to vehicle-treated controls, MOR KO mice and wild-type mice that underwent fluoxetine treatment exhibited decreased latencies to feed in the NSF test and they also exhibited lower durations of immobility in the forced swim test.

**Conclusions:** Here, we performed a gene analysis study and found that PENK was significantly upregulated in the dentate gyrus of mice that behaviorally responded to fluoxetine treatment relative to non-responding mice and vehicle-treated controls. It is possible that increased enkephalin is relevant to antidepressant efficacy, and if so, such effects would result from activity at specific opioid receptors. However, the results of these experiments suggest that enkephalin's antidepressant effects are not mediated by MOR because the antidepressant behavioral effects of fluoxetine treatment were present in MOR KO mice. Enkephalin can also bind the delta opioid receptor (DOR), which is also expressed in the hippocampus, so it is possible that the increased PENK expression that was observed here might relate to activity via DORs. Future studies will explore this possibility in DOR KO mice.

**Keywords:** Fluoxetine, Proenkephalin, Mu-Opioid Receptors, Depression, Corticosterone

**Disclosure:** Nothing to disclose.

## T35. Targeted Action of Intranasal Oxytocin on Brain Functional Connectivity in Adults With Autism Spectrum Disorder

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**Background:** Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social communication and social interaction and restricted, repetitive behaviors. A growing body of evidence supports a potential role for intranasal oxytocin (IN-OXT) in improving social functioning in ASD. However, questions remain about the therapeutic effectiveness and the long-term benefits of IN-OXT.

**Methods:** Here, we tested the effects of several doses of IN-OXT (8IU, 24IU, 48IU, placebo) on brain functional connectivity during resting state (rsFC) in adult males with ASD ($N = 32$, ages 18 - 45, $IQ > 70$) in a randomized, double-blind, within-subject design. We hypothesized that IN-OXT affects the rsFC (8 minutes, open eyes) between brain networks that are involved in socio-emotional processes and in particular, the empathy or the salience network. MRI images were acquired on a 3 T Siemens Magnetom Trio TIM scanner, and data analysis was performed using AFNI and FSL software packages.

**Results:** Independent component analysis showed that 48IU of IN-OXT increased the rsFC between the empathy network and temporal, visual and theory of mind networks, as compared to lower doses or placebo. It also decreased the rsFC among executive networks, dorso-lateral prefrontal cortex and visual and temporal areas, including amygdala.

**Conclusions:** These findings provide evidence for the dose-dependent targeted action of IN-OXT on brain function. The results help to define optimized oxytocin-based acute interventions in individuals with ASD and argue strongly for further studies to examine chronic interventions bolstering OXT-mediated neurotransmission as potential therapies for ASD and other conditions with impairments in social functioning.

**Keywords:** Oxytocin, Autism Spectrum Disorders, Dose-Response Analyses, Resting State Functional Connectivity

**Disclosure:** Nothing to disclose.

## T36. The Relationship Between Whole-Brain Functional Connectivity and Autism Spectrum Disorder Symptoms

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**Background:** Previous studies have suggested that aberrant brain functional connectivity in autism spectrum disorders (ASD) may relate to clinical symptoms, such as social-communication deficits and restricted, repetitive behaviors. These observations have largely not been replicable, however, likely due to small sample sizes and varying use of a priori brain regions of interest, which may bias analyses. The current study addresses this by investigating the relationship between functional connectivity in the brain and clinical symptoms of ASD using a data-driven, comprehensive, whole-brain analysis with a large sample size. Utilizing data from the Autism Brain Imaging Data Exchange (ABIDE), a large, multi-site, publicly-available database that includes functional magnetic resonance imaging (fMRI) and phenotypic data for both

individuals with ASD and typically-developing individuals, the association between total scores on the Autism Diagnostic Observation Schedule (ADOS) and intrinsic functional brain connectivity was examined.

**Methods:** The current study utilized data from the ABIDE I data release. Participant data (N = 108 [90 M, 18 F], from 4 sites) meeting the following criteria were included: resting-state fMRI data available (minimum duration of 5 minutes); ADOS scores available; full-scale IQ > 70; data collected at a site with > 10 participants after applying inclusion criteria. Group Independent Components Analysis (GIFT) was used to identify intrinsic brain networks. Functional connectivity was examined by correlating the time series of each individual brain voxel with the time series of each intrinsic brain network. This resulted in a metric summarizing each voxel's connectivity to all brain networks. Distance covariance analysis was then used to investigate the relationship between connectivity at each voxel in the brain (i.e., the level of connectivity between each individual voxel and all networks) and ADOS total score. Results were corrected for multiple comparisons using a cluster-level, non-parametric test.

**Results:** Connectivity was significantly associated with ADOS scores in clusters located in the right anterior insula, middle insula, right temporoparietal junction (TPJ), left fusiform gyrus, middle frontal gyrus, and middle insula ($p < 0.05$, corrected). Six intrinsic brain networks significantly contributed to the associations between ADOS scores and these regions ($p < 0.05$, corrected): the ventral default mode network (vDMN), a language network, a primary visual network, a sensorimotor network, and two salience networks (dorsal anterior cingulate cortex salience network [dSN); anterior insula salience network [aSN]). Associations between connectivity and ADOS scores differed by anatomical region. The relationship between right TPJ connectivity and ADOS score was driven by the vDMN, aSN, and the primary visual network. In the anterior insula region, however, the association between connectivity and ADOS score was influenced by the aSN, dSN, vDMN, and the language network. In the left fusiform gyrus, the relationship between connectivity and ADOS score was influenced by primary visual, language, and sensorimotor networks. As such, the influence of network connectivity on ADOS scores was not uniform, instead varying by neuroanatomical region. To examine the directionality of connectivity associations, the average connectivity between each network and all voxels within each of the three above regions (right TPJ, anterior insula, left fusiform gyrus) was entered as a predictor for ADOS scores in separate linear regression models. In all three regions, ADOS scores were associated with decreased connectivity ($p < 0.001$, uncorrected), albeit with relatively low coefficients of determination ($r2 < 0.2$). Although supportive of hypoconnectivity in ASD, the low fit of the regression lines may suggest that this is a not a simple linear relationship.

**Conclusions:** The current study examined the relationship between functional connectivity and clinical symptoms of ASD, in a whole-brain, voxel-level analysis, using fMRI and a novel combination of ICA and distance covariance analyses. By using data from the ABIDE initiative, a large-scale, multi-site database, in combination with these novel data-driven statistical methods, the study aimed to comprehensively and reproducibly assess how ASD clinical symptoms relate to functional connectivity in the brain. Associations between connectivity and ADOS scores at any given brain region were influenced by multiple networks, which differed depending on the region. Hypoconnectivity was associated with ADOS scores in all major regions and across almost all connections. The current results, however, suggest a somewhat specific, fine-grained contribution of hypoconnectivity to ASD symptoms, given that hypoconnectivity was associated with ADOS scores in specific cortical regions (i.e., not everywhere in the brain) and with connections to specific networks (i.e., not all large-scale networks). These results suggest heterogeneity of connectivity within the brain as it relates to clinical symptoms, perhaps reflecting the clinical heterogeneity of ASD.

**Keywords:** Autism Spectrum Disorder, Functional MRI (fMRI), Intrinsic Functional Connectivity, ABIDE

**Disclosure:** Nothing to disclose.

## T37. Functional Imaging Together With Deep Brain Stimulation in the VTA Reveals D1 vs. D2 Receptor Contributions in the Non-Human Primate

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**Background:** Dopaminergic projections in the brain originate in the ventral tegmental area (VTA) and substantia nigra and can modulate signals along projections to the cortex and striatum. Electrical stimulation in the VTA has been shown to induce motivational/behavioral changes and reinforcement learning, presumably due to dopamine release from cells originating in the VTA (Arsenault, Curr. Biology, 2014; Schluter, PLOS ONE, 2014; Gunduz, Front Neurosc, 2017). During anesthesia, VTA stimulation has been shown to elicit arousal in rodents that can be blocked by D1 antagonists (Taylor, PNAS, 2016). Yet, the relative contributions of dopamine receptor subtypes or potentially other neurotransmitters that drive downstream signal changes due to VTA stimulation are still largely unknown. The purpose of this proof-of-concept study was to determine the underlying receptor-specific mechanisms of acute VTA stimulation by determining dopaminergic receptor subtype contributions and their whole-brain circuit modulation using both functional magnetic resonance imaging (fMRI) and positron emission tomography (PET).

**Methods:** An integrated PET/MRI was used to simultaneously acquire [11 C]raclopride-PET and fMRI data during acute stimulation of an anesthetized baboon (1.5% isoflurane) with a chronic microstimulation implant in the right VTA. In PET/fMRI sessions (n = 8), bolus + infusion [11 C]raclopride dynamic scans were acquired to directly determine D2 receptor (D2R) binding, with stimulation blocks starting at ~35 min into the scan. Stimulation parameters included pulse trains of 200 ms at 100 Hz at a constant current of 1 mA, with repeated inter-stimulation intervals of 1-8 s over a period of 5-30 min. In order to distinguish receptor-specific contributions, fMRI sessions (n = 5) were designed to stimulate before and after a blocking dose of the D1 receptor (D1R) antagonist SCH23390 (0.03-0.09 mg/kg i.v.) or the D2R antagonist prochlorperazine (0.1 mg/kg i.v.). In these sessions, stimulation was administered as a block design with inter-stimulation intervals of 2 s that lasted for 15 s and alternated with 93 s of rest for a total stimulation time of 10 min. Gradient-echo echo planar imaging data were acquired continuously during stimulation and ferumoxytol was used as a contrast agent. fMRI data were analyzed with a GLM and converted into cerebral blood volume (CBV) changes. PET data were analyzed with a reference tissue model (Ichise, JCBFM, 2003).

**Results:** fMRI data due to stimulation showed robust positive CBV signals in the right (implanted) hemisphere, with the largest signals up to 6% CBV observed in right caudate, nucleus accumbens and putamen ($P = 0.0001$, with significance reached even at the single session level due to the large effect size). Comparisons of the stimulation-induced fMRI signal before and after pharmacological blocking of D2R resulted in a significant signal increase in all previously activated regions. After D2R blocking, the %CBV signal was on average 1.3 (stdev 0.2) times larger than in the pre-drug stimulation condition in putamen,

caudate and nucleus accumbens. Additional activation was observed in the prefrontal cortex and the contralateral side of the striatum. Pharmacological blocking of D1R showed a signal decrease by a factor of 0.5 (0.1) on average in the previously activated regions. Baseline PET binding potential (BPnd) values across sessions were 3.8 (stdev 0.3) (putamen), 2.8 (0.3) (caudate) and 2.1 (0.1) (nucleus accumbens), with no significant lateral differences. [11 C]raclopride binding during stimulation showed small changes in BPnd in the right nucleus accumbens, equivalent to a 10% change in occupancy. Finally, during intense stimulations of 2 s or faster that were continuous for 5 min or longer, indices of arousal were observed in four repeated sessions. Robust increases in blood pressure, end-tidal CO2, and a decrease in breaths per minute were observed that were clearly linked to stimulation intervals in time.

**Conclusions:** This study showed that microstimulation in the VTA resulted in robust positive CBV changes. Displacements of [11 C]raclopride were found in the nucleus accumbens on the implanted side but are small and possibly close to the limit of detection with PET imaging. Therefore, we investigated whether fMRI changes were driven by dopamine through a set of blocking studies with D1R and D2R antagonists at doses that have achieved > 90% occupancy in separate studies. The observed CBV increase after blocking D2R suggests that only part of the CBV signal (23% on average in putamen, caudate, nucleus accumbens) is likely to be D2R-mediated, with the CBV increase demonstrating a block of inhibitory D2R signaling. In concordance with that, the D1R block resulted in a CBV decrease, demonstrating a partial excitatory D1R contribution (45% on average, including an estimated inhibitory D2R signal) that can be suppressed. Yet, the fact that the overall CBV signal remains positive even with a D1R block suggests that a part of the remaining signal is driven by a non-dopaminergic neurotransmitter/receptor. Finally, the indication the arousal can be induced by strong VTA stimulation is an interesting novel observation in non-human primates that warrants further investigation and is consistent with recent findings in rodents4. Overall, these results demonstrate that both D1R and D2R signaling are likely to contribute to DBS-induced functional signaling, but with other neurotransmitters likely playing an important role for VTA stimulation.

**Keywords:** Deep Brain Stimulation, Dopamine Receptors, Functional MRI (fMRI), PET Imaging, Non-Human Primate

**Disclosure:** Nothing to disclose.

### T38. Neurocognitive Markers of Childhood Abuse in Individuals With PTSD in the Intrust Clinical Consortium

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**Background:** Background: PTSD has been associated with neurocognitive abnormalities, but to date few studies have evaluated the contribution of early life experiences to neural and cognitive outcomes. Childhood maltreatment is common among individuals with PTSD and is proposed to catalyze stress-related biobehavioral changes that have lasting impacts on brain structure and function in adulthood. Thus, examining the effect of childhood maltreatment may elucidate neurocognitive heterogeneity observed in earlier studies of individuals with PTSD.

**Methods:** Methods: The current study examined brain morphology and neuropsychological differences in individuals with PTSD with low and high self-reported childhood abuse as compared to healthy controls. Thirty-six individuals with PTSD and low childhood abuse, thirty-one individuals with PTSD and elevated childhood abuse, and 114 healthy controls completed an MRI scan and neuropsychological testing as part of the INjury and TRaUmatic Stress Clinical Consortium (INTRuST) imaging repository. Brain volume and cortical thickness were analyzed using Freesurfer software. Univariate analysis of variance was conducted to examine group differences on continuous demographic, clinical, and neuropsychological domains; chi squared was used for categorical variables. Neuropsychological test analyses controlled for age and sex. Analysis of variance was conducted on volumetric and cortical thickness data with hemisphere entered as a within-subjects factor to examine potential interaction effects of maltreatment group on lateralization. MRI analyses controlled for age, sex, and scanner.

**Results:** Results: Childhood abuse group was associated with cortical thickness in the orbitofrontal/superior temporal cortex ($F(2,174) = 4.9$, $p = 0.01$, $\eta 2 = .05$), inferior frontal gyrus ($F(2,174) = 5.09$, $p = 0.01$, $\eta 2 = .06$), dorsolateral prefrontal cortex ($F(2,174) = 3.58$, $p = 0.03$, $\eta 2 = .04$), cuneus ($F(2,174) = 11.05$, $p < 0.001$, $\eta 2 = .11$), insula ($F(2,174) = 4.00$, $p = 0.02$, $\eta 2 = .04$), rostral anterior cingulate ($F(2,174) = 11.05$, $p = 0.04$, $\eta 2 = .04$), and medial prefrontal cortex ($F(2,174) = 5.61$, $p < 0.01$, $\eta 2 = .06$). In all cases, the mean value for cortical thickness was highest in the control group, followed by the PTSD-A group, followed by the PTSD + A group. Childhood maltreatment group was associated with performance on verbal memory ($F(2,166) = 6.24$, $p = .002$), processing speed ($F(2,165) = 8.24$, $p < .001$), and executive functioning ($F(2,152) = 5.33$, $p = .006$). In all cases, test performance was highest in the control group, followed by the PTSD-A group, followed by the PTSD + A group.

**Conclusions:** Conclusions: Our data indicated that while PTSD was associated with abnormalities in medial and dorsal PFC, insula, temporal, and occipital regions considered critical to anxiety-related processes, these abnormalities were amplified in those with childhood abuse history. The associations between childhood abuse experiences and neurocognitive outcomes converge with earlier data showing that early life experiences affect stress-sensitive brain structures in the prefrontal cortex and cingulate and are linked to lower neuropsychological performance, which may contribute to the cognitive and affective clinical presentation of these individuals. These data suggest there are neurocognitive features associated with childhood abuse within PTSD populations and highlight the need to assess developmental history of maltreatment when examining biomarkers in this disorder.

**Keywords:** PTSD, Childhood Maltreatment, Structural MRI, Neuropsychology

**Disclosure:** Nothing to disclose.

### T39. Two-Photon Interrogation of a Sensory Biomarker of Schizophrenia

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**Background:** Cortical processing of sensory events is significantly influenced by context. For instance, repetitive, or contextually redundant, stimuli elicit attenuated responses in primary visual cortex (V1), while unexpected or "deviant" stimuli elicit augmented responses. This contextual modulation of sensory processing is likely a fundamental function of cortical circuits, yet a generalized understanding of how it is computed is still missing. Importantly, context processing is significantly altered in individuals with

schizophrenia (SZ; e.g. reduced "mismatch negativity"), potentially relating to core pathophysiology and symptomatology.

**Methods:** We measured neuronal activity in cortical volumes in V1 of awake mice (n = 20, male and female) using dual color and holographic two-photon calcium imaging to explore how local, intralaminar, and inter-areal circuits process deviant and redundant events. A visual "oddball" paradigm was employed wherein full-field square-wave stimuli of 2 possible orientations (e.g. 45 vs 135 degrees) were presented with varying regularity (88% redundant orientation; 12% deviant or "oddball" orientation). Responses in this paradigm were compared to a "standard" context wherein stimuli of 8 possible orientations were randomly presented. As described (Hamm and Yuste, 2016), stimulus-specific adaptation (i.e. reduced neural firing to redundant; via t-tests) and deviance detection (i.e. enhanced firing to deviants) were observed at the population level, after averaging over all neurons.

**Results:** Interestingly, cluster analysis indicated the presence of 3 distinct groups of neurons: 40% showing a preference for standard contexts ("general adapting"), 40% showing a preference for deviant contexts ("deviance detectors") and 20% lacking contextual modulation. Only the first two subgroups showed orientation selectivity. These context-specific subgroups of cells were present in layers 2-5 of cortex, although "deviance detectors" were twice as common in layer 2/3. By imaging the activity of axons originating from distal regions, we found that these context-driven preferences are i) absent in bottom-up inputs from dLGN thalamus, and ii) present in top-down projections from PFC (area Cg). Optogenetic suppression of PFC inputs to V1 disinhibited neuronal responses, affecting context processing at a gross level (difference decreased > 2 standard deviations; paired t-tests, $p < .01$), but left subgroup preferences intact.

**Conclusions:** Our results suggest that cortical ensembles can independently code for contextual information, such as stimulus novelty. This underscores the relevance of ensembles in SZ pathophysiology, given that ensembles are altered in pharmacological and genetic mouse models of SZ (Hamm et al, 2017).

**Keywords:** Mismatch Negativity, Cortical Circuit Function, Schizophrenia, Cortical Layers, Two-Photon, Optogenetics

**Disclosure:** Nothing to disclose.

### T40. Hippocampal and Thalamic Regulation of VTA Dopamine Neurons and the Reversal of Aberrant Dopamine System Function by the Orexin Receptor Antagonist TCS 1102 in a Rodent Model of Schizophrenia

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**Background:** Psychosis observed in patients with schizophrenia is associated with aberrant dopamine system function and thought to be driven by hyperactivity from the ventral hippocampus (vHipp). The pathway by which the vHipp regulates dopamine neuron activity has been previously demonstrated and involves a glutamatergic projection to the nucleus accumbens (NAc). Additionally, inactivation of the vHipp is sufficient to restore normal dopamine system function in rodent models of the disease. Recent post-mortem studies have identified glutamatergic abnormalities in the NAc of individuals with schizophrenia are likely attributable to alterations in thalamic inputs to this region.

**Methods:** Here we used male rats to examine the regulation of dopamine neuron activity by thalamic and hippocampal afferents in two rodent models of schizophrenia (MAM and Poly I:C) using chemogenetic approaches combined with electrophysiology. We also assessed whether targeting the orexin system, with the antagonist TCS 1102, was sufficient to reverse deficits in dopamine system function and behaviors associated with schizophrenia in the MAM model of the disease. Data was analyzed by one-way or two-way ANOVA and the Holm-Sidak post-hoc, with significance determined at $p < 0.05$.

**Results:** Electrophysiological experiments revealed that activation of either the vHipp or thalamus (specifically the paraventricular nucleus of the thalamus (PVT)) induced a significant increase in dopamine neuron population activity ($p < 0.001$). Additionally, inactivation of the PVT, much like the vHipp, leads to restoration of normal dopamine system function in rodent models of the disease ($p < 0.001$). Chemogenetic studies demonstrate that PVT projections to the NAc were responsible for this regulation of dopamine neuron activity. These data provide evidence that thalamic abnormalities may contribute to aberrant dopamine system function observed in schizophrenia and that the PVT may be a novel therapeutic target. Given that orexin receptors are expressed in the thalamus they may serve as a target for pharmacological manipulation of PVT inputs to the NAc. Indeed, we provide evidence that both systemic and intracranial (PVT) administration of the orexin antagonist, TCS 1102, can normalize aberrant dopamine system function and behaviors associated with schizophrenia in the MAM model of the disease ($p < 0.001$).

**Conclusions:** Collectively these data suggest that the PVT, and potentially the orexin system, may represent a novel site of intervention for psychosis associated with schizophrenia. Further investigation of the role of the PVT and orexin system in relation to schizophrenia will provide a better understanding of underlying pathophysiology of the disease, ultimately leading to novel sites of therapeutic intervention.

**Keywords:** Paraventricular Nucleus of the Thalamus, schizophrenia, Orexin System

**Disclosure:** Nothing to disclose.

### T41. In-Vivo Alpha-7 Nicotinic Acetylcholine Receptor Availability and Relationship to Cognition in Schizophrenia

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**Background:** Converging lines of evidence from genetic and postmortem studies suggest a role for alpha-7 nicotinic acetylcholine receptor (α7-nAChR) in the pathophysiology of schizophrenia (SZ). Genetic studies, including association, linkage and copy-number variation (CNV) studies demonstrate an association between α7-nAChR gene (CHRNA7) and SZ. Additionally, α7-nAChR mRNA expression has been found to be regulated by neuregulin-1 gene, a gene that is associated with regulating neuronal excitatory/inhibitory balance. Postmortem studies show reduced α7-nAChR protein expression in in the dentate gyrus, hippocampal CA3 region, cingulate, orbitofrontal cortex and reticular nucleus of thalamus in SZ. The availability of [18 F]-ASEM, a positron emission tomography (PET) ligand with high specificity for α7-nAChRs provides an opportunity to examine α7-nAChR in-vivo in humans.

**Methods:** Male schizophrenia subjects (SZ) (n = 6) (Mean age = 44.1 ± 10.9), and age-, gender- matched healthy controls (HC) (n = 6) (Mean age = 44.3 ± 11.0) underwent [18 F]-ASEM PET imaging using High-resolution Research Tomograph (HRRT) and 3 T structural MRI for coregistration. Arterial sampling was used to measure plasma input function. Volume of distribution (VT) was estimated using multilinear analysis (MA1). Verbal memory was

assessed using the Rey Auditory Verbal Learning Task (RAVLT). Electroencephalography (EEG) was acquired during the RAVLT using a paradigm designed to capture EEG processes during passive listening, memory encoding, and recognition.

**Results:** SZ subjects had lower nAChR availability (VT) compared to the HC group in the hippocampus (18%) ($p = 0.04$), and anterior cingulate cortex (ACC) (15%) ($p = 0.06$). Lower hippocampal α7-nAChR availability correlated with performance on verbal recall (memory) measured by the RAVLT ($r = 0.56$). Encoding related theta (θ) power at electrode FCz (where power was the largest) was positively correlated with α7-nAChR availability in both the hippocampus ($r = 0.90$, $p = 0.035$) and frontal cortex ($r = 0.89$, $p = 0.046$); and was positively correlated (trend level) with performance on the RAVLT (short delay recall; $r = 0.65$).

**Conclusions:** Our results provide preliminary evidence of regional decrease in nAChR availability in SZ. The association between nAChR availability, cognitive test performance and EEG measure of encoding suggests the relevance of these receptors in the pathophysiology of cognitive impairment associated with schizophrenia.

**Keywords:** Alpha-7 Nicotinic Acetylcholine Receptor, PET Imaging, Cognition, Psychotic Disorders

**Disclosure:** Nothing to disclose.

## T42. Functional Connectivity of the Globus Pallidus in Patients With First-Episode Schizophrenia: Implications for Outcomes

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**Background:** Abnormalities in the connectivity between cortex and basal ganglia have been reported in schizophrenia. Importantly, abnormal pallidal functioning has been found to be associated with impairments in learning, cognitive deficits, and akinesia, all of which are known to be abnormal in schizophrenia. However, the role of the globus pallidus (GP) in this disorder remains unclear. The following study examined functional interactions of the GP in patients with first-episode schizophrenia (FES).

**Methods:** Resting state fMRI scans were obtained on a 3 T scanner from patients with FES ($N = 43$, 12 F, 12-35 years old, mean = 22.92) and age- and sex-matched healthy controls ($N = 24$, 10 F, 16-28 years old, mean = 22.28). All participants completed cognitive testing at baseline. Patients were assessed clinically at baseline and six-month follow-up via the Strauss-Carpenter Outcome Scale (SCOS). Regions of interest (ROIs) in bilateral internal and external GP structures were drawn based on anatomical location. Parcellation of the GP was conducted using an independent components analysis and one seed within each structure was created. Whole-brain resting-state connectivity maps were generated from these seeds and group differences in pallidal connectivity were assessed. For a subset of our patients with follow-up SCOS data, functional connectivity was examined between low- and high-functioning patients and controls. Significance was defined voxel-wise at $P < 0.005$, and with cluster correction at $P < 0.05$.

**Results:** Patients showed significantly lower functional connectivity between the left GP interna and the right dorsolateral prefrontal cortex, right caudate, and cerebellum. These findings were not associated with cognitive or clinical measures at baseline. Furthermore, patients with lower overall functioning scores at six months showed significantly lower connectivity between the left GP interna and area predominantly within the salience network, including the dorsal anterior cingulate, and left and right insula. Patients in this group also showed significantly lower connectivity between the right GP interna and the anterior cingulate, right inferior frontal gyrus, and left putamen. No connectivity differences between patients in the higher follow-up score group and controls were observed for any seed.

**Conclusions:** These results provide novel evidence for abnormalities in functional interactions of the GP with key prefrontal cortical regions in first-episode psychosis patients. Our findings also suggest that greater reduction in prefrontal-pallidal connectivity may serve as a biomarker of outcome.

**Keywords:** Functional Connectivity, Globus Pallidus, Schizophrenia

**Disclosure:** Nothing to disclose.

## T43. Minimizing Antipsychotic Exposure in the Treatment of Schizophrenia: A Literature Review and a Cross-Sectional Survey

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**Background:** It is critically important to minimize the exposure to antipsychotics in the maintenance treatment of schizophrenia in light of their dose-dependent side effects, such as extrapyramidal symptoms, cognitive impairment, and cardiac sudden death. However, it is unclear regarding the strategy for successful antipsychotic dose reduction. Moreover, while some patients with schizophrenia remain clinically well without continuous antipsychotic treatment, data on the features of such patients are still scarce and previous reports have not evaluated key elements such as physical comorbidities and functioning.

**Methods:** A systematic literature search for studies examining antipsychotic dose reduction in schizophrenia was conducted in March 2018, using PubMed and Embase with the following search terms: ((antipsychotic or neuroleptic or tranquiliz*) AND (dose or dosage) AND (reduce or reduction or low*-dose or minim* or decrease) AND schizophreni* AND adult). The search was filtered with Humans and English. Successful dose reduction was defined as any significant superiority or no significant difference in relapse rate in the dose reduction group versus maintenance group, or any significant improvement or no significant change in symptom severity between pre- and post-reduction. In case all included studies identified a certain factor for successful dose reduction while a majority of unsuccessful studies did not show that specific factor for unsuccessful dose reduction, it was considered to be a predictor of successful dose reduction.

We also conducted a systematic literature review to identify predictive factors for successful antipsychotic discontinuation in schizophrenia using PubMed (last search; June 2018) with the following search terms: (antipsychotic* or neuroleptic) AND (withdraw* or cessat* or terminat* or discontinu*) AND (schizophreni* or psychosis). Factors associated with a lower risk of relapse, when replicated in two or more studies with a follow-up period of three months or longer, were extracted.

Furthermore, six patients with schizophrenia who were withdrawn from antipsychotics after a 12-year follow-up survey underwent comprehensive assessments including the Positive and Negative Syndrome Scale (PANSS), WHOQOL-BREF, and Barthel Index of Activities of Daily Living (Barthel Index).

**Results:** We identified 36 studies for antipsychotic dose reduction trials. Eighteen studies (50%) were randomized controlled trials, and 20 studies (56%) targeted first generation antipsychotics. Relapse rates or symptom changes were compared

between the dose reduction and maintenance groups in 18 studies, and between pre- and post-reduction in five studies. Among those 23 studies, dose reduction was successful in 19 studies (83%). Study duration of < 1 year, age of > 40 years, duration of illness of > 10 years, and post-reduction chlorpromazine equivalent (CPZE) dose of > 200 mg/day were found to be associated with a successful dose reduction. Subjects who had experienced a clinical deterioration were stabilized by increasing the doses back to the baseline doses.

Systematic literature search for antipsychotic discontinuation identified 37 relevant articles. Mean relapse rate following antipsychotic discontinuation was 38.3% (95%CI = 16.0-60.6%) per year. Factors associated with a lower risk of relapse were being maintained on a lower antipsychotic dose before discontinuation, older age, shorter duration of untreated psychosis, older age at the onset of illness, a lower severity of positive symptoms at baseline, better social functioning, and a lower number of previous relapses.

A cross-sectional survey of six antipsychotic-free patients with schizophrenia revealed that four inpatients were old (mean ± SD, 77.8 ± 4.8 years) and chronically ill (duration of illness, 49.3 ± 12.5 years) with a high PANSS total score (118.0 ± 1.8), physical comorbidities, and low functioning (Barthel Index, 8.8 ± 11.1). By contrast, two outpatients were relatively young (45.0 ± 12.0 years) and clinically in good condition (PANSS total score, 44.5 ± 0.5; Barthel Index, 100 for both). One patient discontinued antipsychotics after having achieved remission, while the other five stopped them due to physical comorbidities.

**Conclusions:** Antipsychotic dose reduction was successfully performed in a majority of the previous studies while the increased risk of relapse after a prolonged follow-up period should be taken into consideration and target doses may need to be set conservatively (e.g. > 200 CPZE mg/day). Special caution should be exercised for younger patients with a relatively short illness duration in reducing the dose of antipsychotics. Although the literature review suggests some predictors for successful antipsychotic withdrawal in patients with schizophrenia, the very limited evidence base and unequivocally high relapse rates following discontinuation must remain a matter of serious debate for risk/benefit considerations. We found a subgroup of patients with schizophrenia who may not need antipsychotics for relapse prevention in our longitudinal follow-up survey. The heterogeneity of this patient population warrants further exploration to elucidate neurotransmitters beyond dopamine as an underlying pathophysiology of schizophrenia.

**Keywords:** Schizophrenia, Antipsychotics, Dose Reduction, Withdrawal

**Disclosure:** Nothing to disclose.

### T44. Chronic Haloperidol Treatment Drives Neuroinflammation in Rats Exposed Prenatally to Maternal Immune Activation

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**Background:** Evidence-based medicine suggests that a subset of schizophrenia cases may be due to neuroinflammation, characterized in particular by microglial activation. However, we lack a basic understanding of the impact of antipsychotic drugs on neuroinflammation. In a preliminary study to address this, we investigated the effects of chronic haloperidol treatment in an established rat model of maternal immune activation (MIA), a known risk factor for schizophrenia and other psychiatric disorders (Meyer U. Biological Psychiatry, 2014). Based on observation that chronic antipsychotic exposure increases microgliosis in naive rats (Cotel et al., European Neuropsychopharmacology; 2015), we hypothesized that chronic haloperidol will interact with MIA to drive central microgliosis.

**Methods:** Male offspring (age: post natal day 90) from control (CON: 0.9% saline, i.v.; GD15; n = 5) and MIA-exposed dams (poly (I:C); 4 mg/kg i.v. GD15; n = 5) were selected at random and treated with either the dopamine receptor antagonist (D2) haloperidol (HAL: 0.5 mg/kg/d s.c.) or common vehicle (VEH: β-hydroxypropylcyclodextrin, 20% wt/vol, acidified by ascorbic acid to pH 6) for 28 days. There were four treatment arms: CON/VEH; CON/HAL; MIA/VEH and MIA/HAL (all group sizes, n = 10 offspring). The dose of haloperidol and route of administration have been previously shown to result in clinically comparable plasma drug levels and pharmacokinetics (Vernon et al., Biological Psychiatry, 2012). At the end of treatment, animals were culled and perfused transcardially with 4% PFA. Fixed brain tissues were then sectioned (1 in 12 series, 40 um) and stained for Iba1 and GFAP to assess microglia and astrocytes, respectively (Cotel et al., European Neuropsychopharmacology, 2015). Adjacent tissue sections were stained for both Iba1 and translocator protein (TSPO), a cross-species putative neuroimaging marker of gliosis (Notter et al., Molecular Psychiatry, 2018). Density and soma size of Iba1 + microglia were quantified using unbiased stereology in the corpus striatum, which expresses the highest density of dopamine D2 receptors and is a primary site of antipsychotic drug action. In parallel, GFAP + astrocytes were quantified by optical density measurements (area fraction) in the same region. Iba1 + /TSPO + microglia were quantified in the striatum using confocal microscopy to assess the density of Iba1-TSPO double-positive cells. Data for microglia and astrocytes were analyzed using 2 × 2 ANOVA in SPSS, with main effects of prenatal (CON vs. MIA), postnatal (VEH vs. HAL) and pre x post-natal treatment interactions. Post-hoc Bonferroni corrected t-tests were performed when p(ANOVA) < 0.05 and effect sizes for ANOVA are given (η2).

**Results:** In the striatum, a significant main effect of prenatal treatment ($F[1,32] = 18.09$; $p < 0.001$; $\eta2 = 0.5$) but not post-natal treatment ($F[1,32] = 2.13$; $p > 0.05$; $\eta2 = 0.13$) were found for Iba1 + microglia density. Notably, however, a significant prenatal x postnatal treatment interaction was also found ($F[1,32] = 5.15$; $p < 0.05$; $\eta2 = 0.22$). Post-hoc testing on this interaction confirmed a significant increase in Iba1 + density in POL-offspring treated with HAL as compared to VEH ($p < 0.001$). Iba1 + soma sizes were also significantly affected by both prenatal ($F[1,32] = 88.4$; $p < 0.001$; $\eta2 = 0.83$) and post-natal treatment ($F[1,32] = 17.3$; $p < 0.01$; $\eta2 = 0.55$) and there were significant pre x postnatal treatment interactions ($F[1,32] = 11.6$; $p < 0.01$; $\eta2 = 0.39$) such that Iba1 + soma sizes were significantly higher in MIA/HAL treated animals relative to all other groups. For GFAP + astrocytes in the striatum, there were main effects of both prenatal treatment ($F[1,32] = 14.9$; $p < 0.01$; $\eta2 = 0.45$) and postnatal treatment ($F[1,32] = 24.0$; $p < 0.001$; $\eta2 = 0.63$) but no significant interaction. The density of Iba1 + /TSPO + microglia were unaffected by either prenatal or postnatal treatment, with no significant main effects or interaction (all $p > 0.05$).

**Conclusions:** Our data suggest three new findings relevant for the study of neuroinflammation in schizophrenia. First, chronic postnatal haloperidol treatment interacts with prenatal exposure to MIA to drive an Our data suggest three new findings relevant for the study of neuroinflammation in schizophrenia. First, chronic postnatal haloperidol treatment interacts with prenatal exposure to MIA to drive an increase in Iba1 + density and soma size in the rat striatum, suggestive of microglial activation. Second, both MIA and HAL treatment alone induce GFAP + astrocytosis, but these do not significantly interact. Third, despite the increase in microglia density in MIA/HAL treated animals, this is not

accompanied by a detectable increase in TSPO, a putative non-invasive imaging marker of gliosis. This is consistent with human PET studies reporting no effect of antipsychotic medication on TSPO radioligand binding in schizophrenia patients (Plavén-Sigray et al, Biological Psychiatry). Nonetheless, although preliminary, our data suggest antipsychotic treatment increases brain microgliosis in MIA-exposed offspring. It may be hypothesized that haloperidol could exacerbate microglial activation in those schizophrenia patients where neuroinflammation may be an underlying cause, which may remain undetectable with current in vivo neuroimaging tools. Further studies are now required to determine the molecular profile of these microglia to establish whether microglial activation is detrimental or beneficial in this rat model.

**Keywords:** Antipsychotic, Schizophrenia, Translocator Protein, Microglia, Neuroinflammation

**Disclosure:** Nothing to disclose.

### T45. Activation of the Acetylcholine Muscarinic M1 Receptor Modulates Nucleus Accumbens Dopamine Release: Implications for Motivational Deficits

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**Background:** Currently approved antipsychotics are efficacious in treating positive symptoms of schizophrenia in many patients, however, offer little to no benefit for negative or cognitive symptoms, and are associated with a number of adverse side effects. Thus, there is a critical need to develop fundamentally new approaches for treating schizophrenia that provide improved efficacy and induce fewer adverse effects than current medications. In recent years, intense translational efforts suggest that highly selective positive allosteric modulators (PAMs) of the M1 muscarinic acetylcholine receptor (mAChR) may provide a novel approach for cognitive symptoms of schizophrenia. The negative symptoms of schizophrenia are associated with reduced nucleus accumbens (NAc) dopamine (DA) release and subsequent reduction in motivated behavior. Because M1 is highly localized in the NAc and has been shown to regulate DA release, we hypothesize that activation of M1 may be efficacious for the treatment of negative symptoms.

**Methods:** NAc core DA release was assessed by ex vivo or in vivo fast scan cyclic voltammetry. Electrically evoked DA release was obtained from coronal sections via ex vivo recordings. Five-minute application of VU0364572 (100uM) occurred either in the absence or presence of Go 6983 (50 nM). For in vivo recordings, a twisted, bipolar, stimulating electrode was implanted into the ventral tegmental area (VTA) and a carbon fiber working electrode into the NAc core. Biphasic current pulses were applied for 2 s in the VTA to evoke DA release in the NAc core following administration of vehicle, haloperidol, or haloperidol plus VU0364572. Motivated behavior was tested in either a traditional progressive ratio schedule or a concurrent fixed-ratio chow task, where mice have the option to obtain a highly valued reward that requires more effort or approach and consume a less preferred reward that requires no work.

**Results:** Compared to littermate controls, global M1 knockout mice have decreased release and require higher stimulation intensities to evoke DA (two-way ANOVA, $p < 0.01$). Consistent with these findings, bath application of the atypical agonist VU0364572 increases NAc core DA release (t-test, $p < 0.001$) and increases motivated behavior compared to vehicle-conditions (repeated measures ANOVA, $p < 0.001$). These increases in DA release observed following activation of M1 are dependent on protein kinase C (PKC), as incubation of a PKC-inhibitor attenuates increases in DA release. Excitingly, the reductions in NAc DA release observed following administration of the DA D2 antagonist, haloperidol, can be fully attenuated by VU0364572 (one way ANOVA, $p < 0.01$) and can attenuate haloperidol-induced shifts in effort-related choice behavior (repeated measures ANOVA, $p < 0.001$). Examination of tau, the decay constant, suggests that administration of haloperidol plus VU0364572 changes reuptake kinetics of the dopamine transporter (DAT; one-way ANOVA, $p < 0.01$).

**Conclusions:** M1 receptors in the NAc core play an important modulatory role on DA; whereby, activation of M1 increases DA release. These increases in NAc DA release can increase motivated behavior as well as attenuate deficits in effort-related choice observed following administration of haloperidol. Taken together these findings suggest that activation of M1 may be therapeutic for the negative symptoms of schizophrenia.

**Keywords:** M1 and M4 Muscarinic Receptors, Motivation, Schizophrenia Negative Symptoms, Dopamine Release

**Disclosure:** Nothing to disclose.

### T46. Divergent Effects of First and Second Generation Antipsychotics on Neural Ensemble Activity in Striatum

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**Background:** A positive clinical outcome in schizophrenia requires matching patients with the optimal antipsychotic drug (APD), often through trial and error. A major obstacle in this process is minimizing the host of unwanted side effects associated with these drugs. The first generation APD's have a higher propensity than the second generation to induce extrapyramidal side effects such as parkinsonism and dyskinesia. While the distinct pharmacology of these two APD classes likely accounts for this difference, whether the two classes differentially modulate neural coding in movement related brain areas remains unknown. We recently identified multiple neural ensemble signatures of parkinsonism and dyskinesia by using calcium imaging to monitor cellular-resolution D1- and D2-spiny projection neuron (dSPN and iSPN) activity in the striatum of parkinsonian mice. Here we apply these same tools to differentiate between these two APD classes at the level of genetically-specified neural ensemble activity.

**Methods:** We used miniaturized fluorescence microscopes to selectively image dSPN or iSPN calcium activity in the dorsomedial striatum of freely moving mice. We rendered the mice hyperdopaminergic by administering them amphetamine. We then tested the ability of receptor-occupancy matched dosages of a first and second generation APD (haloperidol and aripiprazole, respectively), to reverse the neural ensemble dynamics evoked by excess dopamine.

**Results:** As is well established, both aripiprazole and haloperidol dose-dependently reversed amphetamine-induced hyperlocomotion. Calcium activity was enhanced in dSPNs and reduced in iSPNs by amphetamine, consistent with classical basal ganglia models. On amphetamine, haloperidol did not affect the rates of activity in either cell type, while aripiprazole enhanced the levels of activity in both dSPNs and iSPNs. Additionally, amphetamine enhanced the spatiotemporal coordination of activity iSPNs, but abolished this facet of activity in dSPNs. Neither drug restored the normal spatial structure of dSPN co-activity, while both attenuated the augmented proximal cell co-activity in iSPNs.

**Conclusions:** The distinct extents to which these two classes of APD's reverse the neural ensemble dynamics associated with a hyper dopaminergic state may explain some of the well-documented differences in their side-effect profiles. The neural dynamics described here provide a more robust readout of the effects of these two antipsychotics than behavioral measures alone and may provide a platform for developing novel therapies with greater efficacy and more favorable side-effect profiles.

**Keywords:** Schizophrenia, Antipsychotics, In Vivo Calcium Imaging, Dorsal Striatum

**Disclosure:** Nothing to disclose.

### T47. Sleep Measures and Their Inflammatory and Clinical Correlates in Individuals With Schizophrenia and Bipolar Disorder

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**Background:** Sleep disturbances are central to many psychiatric disorders, including schizophrenia (SZ) and bipolar disorder (BD), with clear implications for cognition, brain health and aging. Although the precise mechanisms are unclear, sleep disturbances are thought to result in increased inflammation, e.g., increased levels of C-reactive protein (CRP), interleukin(IL)-6 and Tumor Necrosis Factor-α (TNF-α). However, there is limited understanding in persons with psychotic disorders, e.g., SZ and BD, of how sleep is associated with inflammatory mechanisms and key outcomes. While sex differences in sleep and inflammation have not been extensively examined in persons with SZ or BD, research in the general population has reported that sex differences exist for both sleep and inflammation; and that sex may moderate the sleep-inflammation relationship.

We present cross-sectional data from ongoing longitudinal studies of SZ, BD and non-psychiatric comparison (NC) subjects. Our hypotheses were: 1) Relative to NCs, persons with SZ and BD would have worse sleep disturbances and elevated levels of pro-inflammatory and reduced levels of anti-inflammatory biomarkers. 2) Sleep disturbances would be associated with worse inflammatory marker levels in the SZ, BD and NC groups, while accounting for relevant demographic, clinical, and cognitive variables. 3) Sex may influence the sleep-inflammation relationship.

**Methods:** The sample included 144 subjects with SZ (DSM-IV-TR criteria), 35 subjects with BD (DSM-IV-TR criteria), and 191 NCs (age range 26 to 65 years). We examined self- reported sleep disturbances (sleep quality and duration). Blood-based systemic inflammatory markers included CRP, IL-6 and TNF-α. Cognitive measures included executive functioning (Delis-Kaplan Executive Function System), overall cognition (MATRICS cognitive composite), and subjective cognitive complaints (Telephone Interview for Cognitive Status - modified.) Group differences were examined with ANOVAs and follow-up t-tests. Within the SZ and BD groups, the relationship of sleep and other clinical predictors to inflammatory marker levels were examined using general linear models with inflammatory marker concentrations as the dependent variable and included measures of sleep quality, age, sex, depressive symptoms, executive functioning, overall cognition, and subjective cognitive complaints as the predictor variables. In the SZ group, we also included positive and negative symptoms as well as daily antipsychotic dose. Sleep quality was included in each model, and a backward elimination approach was used to trim models for all other factors. Last, we examined the sex differences in inflammation and sleep quality in the SZ, BD, and NC groups. If present, we performed general linear models of sleep quality and gender with and without the sleep x gender interactions.

**Results:** The SZ, BD and NC groups were comparable on age, sex, and race. The BD and NC groups had more years of education compared to the SZ group. The SZ/BD group had worse self-reported sleep quality than the NC group (Cohen's $d = 0.59$). The SZ/BD group had significantly higher levels of CRP ($d = -1.36$), IL-6 ($d = -0.31$), and TNF-α ($d = -0.62$) compared to NCs.

While there were no sex differences on sleep or inflammatory measures in the NC and BD groups, women with SZ were more likely to have moderate-poor sleep quality (76% vs. 63%, $X2 = 9.0$, $p = 0.01$) and elevated mean levels of hs-CRP and IL-6 (5.6 ($SD = 6.0$) vs. 4.3 ($SD = 10.1$), $t142 = 2.84$, $p = 0.005$, $d = 0.47$; 1.4 ($SD = 1.3$) vs. 1.1 ($SD = 1.1$), $t133 = 2.19$, $p = 0.03$, $d = 0.38$) compared to men with SZ. There was no significant effect of sleep quality x gender interactions for CRP and IL-6.

Among persons with SZ, sex, age, cognitive complaints and sleep quality were significantly associated with CRP levels ($d = 0.55$, 0.40, 0.38, and 0.37, respectively), such that female sex, older age, worse cognitive complaints and lower sleep quality were associated with higher levels of CRP. Executive functioning and sleep quality were significantly associated with IL-6 levels ($d = 0.70$ and 0.35, respectively), such that worse executive functioning and lower sleep quality were associated with higher levels of IL-6.

Among persons with BD, executive functioning and sleep quality were significantly associated with IL-6 levels ($d = 3.1$ and 1.7, respectively), such that lower sleep quality and worse executive functioning were associated with higher levels of IL-6. Sex was not associated with inflammatory marker levels and sleep quality in the BD group.

**Conclusions:** Self-reported sleep disturbances and increased inflammation are associated in persons with psychotic disorders. Levels of inflammation and sleep disturbances were sex-dependent (women > men) in the SZ group, but not the BD group. While these cross-sectional data support our hypotheses, longitudinal examination of the sleep-inflammation links, their contribution to clinical outcomes, the relationship with objective sleep measures and sex-specific factors is warranted. This study is continuing, and updated results will be presented at the meeting.

**Keywords:** Psychotic Disorders, Inflammation, Cognition

**Disclosure:** Nothing to disclose.

### T48. Dysregulation of the Nucleus Tractus Solitarius-Central Amygdala Circuit With Acute and Chronic Ethanol Exposure

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**Background:** Alcohol dependence is a complex disorder characterized by neuroadaptive changes in specific brain regions and circuits that promote adverse behavioral outcomes associated with alcohol dependence. Previous studies have focused on the role of the central amygdala (CeA) in the context of the extended amygdala circuitry, however, CeA connections with other brain regions can also influence CeA responsivity to ultimately alter behavior. One region that forms connections with the CeA is the Nucleus Tractus Solitarius (NTS). Although the CeA has been extensively implicated in the development of alcohol dependence, the role of brainstem input into the CeA and signaling between the NTS and CeA in alcohol-induced circuit pathology remains unclear. The NTS receives sensory input from the periphery and acts as an integrative autonomic center. The CeA confers emotional relevance to internal and external sensory

input. Thus, the NTS - CeA circuit represents a potential conduit by which peripheral state can influence emotional reactivity and potentially behavior. In that context, the NTS - CeA circuit may also represent an important target for acute and chronic ethanol and an understudied source of circuit dysfunction that alters drinking behavior.

**Methods:** Adult male and female Sprague Dawley rats were used for all experiments. Projection neurons were identified with fluorescent microspheres and/or pseudorabies virus. Immunohistochemistry experiments identify co-expression of cell markers and the immediate early gene c-Fos in defined neuronal populations. Electrophysiological recordings of spontaneous inhibitory postsynaptic currents (sIPSCs) and cell-attached firing were performed in NTS neurons. Repeated intragastric administration of 5 g/kg EtOH or H2O (equal volume) was performed once daily for 14 days followed by 24 h withdrawal to examine the consequences of chronic ethanol exposure. Intracranial injection of adeno-associated virus (AAV) encoding a Gi-coupled (hM4Di) Designer Receptor Exclusively Activated by Designer Drug (DREADD) under the human synapsin (hSyn) promoter and the fluorescent marker mCherry or a control AAV expressing EGFP was injected into the NTS and used to chemogenetically alter the activity of infected NTS neurons. Two bottle choice intermittent access to 20% v/v ethanol or water was performed to measure voluntary alcohol consumption by interval over a 24-hour period in control and experimental conditions. Data are presented as mean ± standard error. Statistical significance was determined using paired or unpaired t-tests, or two-way ANOVAs with posthoc tests where appropriate. In all cases, $p < 0.05$ was set as the criterion for statistical significance.

**Results:** NTS neurons that project to the CeA were identified by monosynaptic retrograde transport of fluorescent microspheres injected into the CeA. CeA-projecting NTS neurons were found to have significantly different membrane properties as compared to unlabeled NTS neurons. The differential membrane properties of CeA-projecting NTS neurons were consistent between NTS neurons from male and female rats. There was some overlap between CeA-projecting and gastric-projecting NTS neurons. Focal application of acute ethanol (44 mM) onto CeA-projecting NTS neurons resulted in increased phasic and tonic inhibition as measured by an increased frequency of sIPSCs and an increase in holding current, respectively, and a significant reduction in firing. Chronic intragastric ethanol exposure did not alter membrane properties in CeA-projecting NTS neurons but did result in increased global activity in the NTS as measured by c-Fos. There was an increase in phasic inhibition and a decrease in tonic inhibition in CeA-projecting NTS neurons following chronic ethanol exposure as well as an increase in baseline firing rate. Rats were allowed to voluntarily consume ethanol for six weeks on an intermittent access schedule where bottles were available Monday, Wednesday and Friday for a 24-hour period. Rats were intracranially injected with an AAV-hSyn-hM4Di containing mCherry or a control vector (AAV-EGFP). DREADD expression and function was validated by cellular recordings in NTS neurons from a separate cohort of rats. Voluntary ethanol and water drinking were measured following administration of the ligand CNO (3 mg/kg) or vehicle in AAV-hSyn-hM4Di and AAV-EGFP control rats. CNO did not alter ethanol drinking in AAV-EGFP control rats, but significantly reduced ethanol consumption in AAV-hSyn-hM4Di rats during the first hour after bottle presentation. Water consumption was not altered in either group.

**Conclusions:** These results demonstrate that acute ethanol alters the activity of NTS neurons that project to the CeA and that chronic ethanol exposure produces long-lasting changes in basal NTS-CeA activity. Chemogenetically inhibiting the activity of the NTS in rats following chronic ethanol exposure reduces voluntary drinking behavior. Collectively, these findings suggest that the NTS-CeA circuit may be an important component of alcohol-related circuit dysfunction that contributes to the pathological behaviors underlying alcohol dependence.

**Keywords:** Alcohol, Brainstem, GABA, DREADDs, Drinking

**Disclosure:** Nothing to disclose.

### T49. Cellular Specificity of Matrix Metalloproteinase Activation on Accumbens Medium Spiny Neurons During Heroin Relapse

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**Background:** Heroin abuse is a leading cause of drug overdose-related deaths in the United States, highlighting a need for further research elucidating effects of maladaptive neuroadaptations following prolonged heroin use. Activation of the tetrapartite synapse in the nucleus accumbens core (NAcore), which comprises of pre- and postsynapse, astrocytic processes, and surrounding extracellular matrix (ECM), has been linked to increased relapse vulnerability. Specifically, degradation of the ECM by activated matrix metalloproteinases (MMPs) is involved in extracellular synaptic remodeling both constitutively and transiently. Following chronic cocaine self-administration, cocaine-extinguished rats exhibit enduring increases in MMP-2 activity in NAcore compared to controls, and MMP-9 activity is transiently increased during cued reinstatement. Interestingly, heroin-extinguished rats do not show constitutive MMP activity, however, transient increases were elicited after 15 mins of cued heroin seeking. Although increases in MMP-2,9 fluorescence can be localized to the soma and dendritic processes of medium spiny neurons (MSNs) in the accumbens, it is unknown which specific cell types harbor changes in MMP activity under heroin-extinguished and cued reinstatement conditions.

**Methods:** We used an AAV cre-dependent mCherry virus to transfect accumbens MSNs in male and female D1 and D2 cre-dependent rats ($n = 13$/cell type) and measured the colocalization of activated MMP-2,9 after FITC-gelatin microinjection under extinguished and reinstated conditions. Statistical analysis performed for these studies were one-way ANOVA.

**Results:** For D1 MSNs, we observed increased MMP-2,9 colocalization with dendritic surfaces in reinstated animals compared to both yoked saline controls and heroin-extinguished animals ($p < 0.0001$). While D2 MSNs showed increased MMP-2,9 colocalization only in heroin-extinguished animals, but MMP-2,9 colocalization after 15 min reinstatement was reduced to yoked saline levels ($p < 0.01$).

**Conclusions:** These findings reveal how NAcore extracellular matrix signaling underlying constitutive and transient synaptic plasticity relies in part on specific cell-types.

**Keywords:** Matrix Metalloproteinase-9 (MMP-9), Medium Spiny Neurons, Heroin Self-Administration, Extracellular matrix, Synaptic Plasticity

**Disclosure:** Nothing to disclose.

### T50. Epigenetic Priming in the Nucleus Accumbens Underlies Relapse of Cocaine-Associated Behaviors

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**Background:** Substance use disorder, a chronic relapsing neuropsychiatric disease, is characterized by the resilience of drug-seeking even following long periods of abstinence. Drugs of abuse, such as cocaine, are known to cause persistent changes in neuronal function throughout the circuitry regulating motivation, memory, and reward. Underlying these changes in circuit function are maladaptive changes in gene expression and synaptic plasticity caused by repeated drug exposure. Due to the persistence of both drug-seeking behaviors and drug-induced plasticity, the addiction field has implicated various epigenetic mechanisms as targets for drugs of abuse. Of note are the changes in the nucleus accumbens (NAc), a key regulator of cocaine-associated and cocaine-seeking behaviors. Recent work has demonstrated a critical role for histone acetylation in the NAc and has identified key genes critical in the acute and chronic effects of cocaine exposure. Yet, while the role of the NAc in cocaine self-administration has been extensively studied, few studies have evaluated the long-lasting changes within this region contributing to reinstatement of cocaine-seeking. Of note are the adaptations within D1-expressing medium spiny neurons (D1-MSN) of the NAc, known to play a major role in mediating both the acquisition and reinstatement of cocaine-seeking behavior. Recent studies in the Calipari lab have identified a unique gene expression profile in the NAc during cocaine-primed reinstatement, including dysregulation of genes with known roles in synaptic function such as Oprk1, Scn4b, and Homer3. We hypothesize that cocaine self-administration generates a long-lasting epigenetic environment which alters the integration of neural circuit activity within genetically defined cell types in the NAc and, ultimately, driving relapse.

**Methods:** To test this, male and female adult (2 months old) C57BL/6 J mice self-administered cocaine (or saline) on an FR5 schedule of reinforcement at 1 mg/kg/inj. In our laboratory, this has been shown to induce robust self-administration. Mice were first be trained on an FR1 schedule. Once mice met acquisition criteria ( > 15 lever presses, 70% on active lever, 3 consecutive sessions), the reinforcement schedule was increased to an FR2 for 3 days, then to FR5 for the remaining 7 sessions. Animals subsequently underwent a 30-day forced abstinence following which reinstatement was induced by a 10 mg/kg i.p. injection of cocaine or saline. Animals were injected with cocaine or saline and placed back into the operant conditioning chambers. During a 15-minute reinstatement session, both nose pokes were active, but had no programmed consequences. 1 h following the cocaine-primed reinstatement session, animals were sacrificed and NAc tissue was collected. Changes in global histone marks were assessed via western blot. Gene-specific targeted changes in histone marks were assessed via chromatin-immunoprecipitation qPCR.

**Results:** In the NAc of cocaine re-exposed animals, we identified several cocaine-mediated changes to histone marks via western blot. We identified a subset of genes enriched for epigenetic marks previously shown to be dysregulated during reinstatement of cocaine-associated behaviors, including H3S10 phosphorylation, H3K9 acetylation, and H3K14 acetylation. Moreover, this subset of phosphoacetylation rich genes coincides with genes dysregulated in the NAc during cocaine-primed reinstatement.

**Conclusions:** The NAc has been extensively studied for its role in regulating reward and drug-associated behaviors. The results of this study provide evidence for long-lasting drug-induced changes to the epigenome. In addition, we provide data linking these changes in epigenetic state to changes in cocaine-induced gene expression that make animals vulnerable to relapse. Future studies will identify a causal link between changes to epigenetic gene regulation and NAc circuit function during relapse-like behaviors.

**Keywords:** Nucleus Accumbens, Neuroepigenetics, Cocaine, Cocaine Reinstatement and Taking, Cocaine Self-Administration

**Disclosure:** Nothing to disclose.

## T51. Cross-Sectional and Longitudinal Assessments of Incubation of Cue-Induced Drug Craving in Cocaine-Addicted Individuals: Preliminary Results

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**Background:** Cue-induced craving is considered as a major contributor to relapse in treatment-seeking individuals with substance use disorders. Animal studies have shown that cue-induced drug-seeking increases (or incubates) during the initial phase of abstinence, presumably reflecting a period of heightened relapse vulnerability. Previously, we have demonstrated the evidence of incubation of cue-induced craving in cocaine addiction using a cross-sectional study design and employing EEG-derived late positive potential (LPP) as a biomarker for cue-induced craving. However, the cross-sectional design of this previous study prohibited delineating the timing of incubation, critical for clinical decisions (e.g., for timely treatment mobilization). In the current study, we used a novel hybrid of a cross-sectional design to validate our previous findings in an independent sample, as well as a longitudinal design spanning 5 follow-ups acquired every 3 months over 12 months of abstinence in the same subjects to inspect within-subject changes in craving with more precise temporal resolution. The study is ongoing and, therefore, only data acquired at baseline and a 3 months follow-up is presented in this abstract [however, the study continues to collect data at additional time-points (6, 9, and 12 months) post abstinence initiation as well as enroll more participants].

**Methods:** Thirty-nine individuals (11 females) abstaining from cocaine (most seeking treatment) were stratified into 3 groups based on their abstinence duration [Group 1 ($n = 20$; Abstinence: 3 – 30 days), Group 2 ($n = 11$; Abstinence: 31 – 60 days), and Group 3 ($n = 8$; Abstinence: 61 – 90 days)]. Seventeen of these 39 participants came back for a 3-months follow-up visit, 10 of whom remained abstinent. At both study visits, all participants completed the Cocaine Craving Questionnaire to report their unprovoked subjective craving. EEG data were recorded as participants passively viewed 30 cocaine pictures and 30 neutral pictures. For each picture, participants also rated the intensity of cocaine 'wanting' (i.e., cue-induced subjective drug wanting) on a 1 – 9 scale, such that a higher rating reflected more cocaine wanting. The LPP elicited by cocaine-related relative to neutral pictures was extracted.

**Results:** Cross-sectional Analyses: Univariate ANOVA on subjective unprovoked craving yielded a significant Group main effect [$F = 4.3$, $p = .02$] and a significant linear contrast [Contrast Estimate = -9.6, $p = .01$], underscoring a gradual decrease in subjective craving with increasing abstinence duration. However, analysis of cue-induced subjective drug wanting did not show a significant decrease from Group 1 to Group 3 ($p = .11$). Replicating our previous results, the univariate ANOVA for LPP revealed a significant Group main effect [$F = 6.6$, $p < .01$] and a significant quadratic contrast [Contrast Estimate = -20.6, $p < .01$], highlighting an initial increase (i.e., incubation) in LPP amplitude from Group 1 to Group 2, before a decline from Group 2 to Group 3. As expected, higher subjective unprovoked craving was positively correlated with higher subjective cue-induced drug wanting across all participants ($r = .5$, $p < .01$) and these subjective measures were not correlated with LPP amplitudes.

Longitudinal Analyses: Longitudinal changes in subjective unprovoked craving, cue-induced subjective drug wanting, and

LPP amplitude from baseline to 3 months follow-up were assessed via paired t-tests, separately for abstainers and relapsers. In abstainers, a decrease in subjective craving from baseline to follow-up did not reach statistical significance ($p = .27$). However, cue-induced drug wanting showed a significant increase (i.e., incubation) from baseline to follow-up ($p = .04$), and the same pattern, although not statistically significant in this small sample size, was also seen in LPP amplitudes ($p = .23$). Relapsers did not show longitudinal changes in subjective craving or LPP amplitudes. However, a trend for an increase in cue-induced drug wanting, similar to that in abstainers, was observed in relapsers ($p = .05$). The incubation of cue-induced subjective drug wanting from baseline to follow-up in both abstainers and relapsers was associated with longer abstinence duration at follow-up ($r = .5$, $p = .08$).

**Conclusions:** To our knowledge the current study is the first to use a hybrid cross-sectional and longitudinal within-subject design to systematically, and using objective measures of brain function, explore incubation of craving in cocaine-addicted humans. The cross-sectional results validated our previous findings of a gradual decrease in subjective craving and of incubation of LPP-assessed cue-induced craving during the first month of abstinence; the longitudinal results showed incubation of cue-induced cocaine wanting (and of LPP amplitudes albeit less strongly) from baseline to 3 months follow-up. Although both experimental designs provide evidence of craving incubation, the inconsistencies between the two highlight the need for and importance of further investigation with a larger sample size. Of note is the dissociation between subjective unprovoked craving and cue-induced craving. These results underscore the need for incorporating measures of cue-induced craving in addition to baseline measures, which are commonly used in clinical settings, to identify precise time-course of personalized craving trajectories and to ultimately provide individualized treatment for preventing relapse.

**Keywords:** Cocaine Addiction, Incubation of Drug Craving, EEG Biomarkers, Longitudinal Study

**Disclosure:** Nothing to disclose.

## T52. Using Genetically Diverse Mouse Populations to Uncover Novel Mechanisms Underlying Circadian Rhythms and Addiction Vulnerability

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**Background:** Circadian rhythm and sleep disruptions are common to many psychiatric disorders, including addiction, and may contribute to the vulnerability of drug abuse and dependence. The genetics and molecular mechanisms underlying these associations remain poorly understood, yet genome-wide and targeted gene association studies have linked circadian gene variants to addiction disorders. While circadian mutant mice (single gene mutations or knock-outs) have been useful for beginning to understand these mechanisms, recent whole genome approaches have revealed many genes with circadian oscillations and/or influence the robustness and timing of the molecular clock. These findings suggest a number of genes may have novel roles in the regulation of cellular and molecular rhythms and addiction vulnerability.

In humans, there is substantial variability of sleep and circadian rhythms (e.g., sleep onset, sleep-wake cycles, molecular rhythms). Moreover, shorter circadian period in molecular rhythms is significantly correlated with illness severity in skin fibroblasts taken from patients with addiction disorders. Similar variations of molecular and behavioral rhythms are observed in fibroblasts from inbred and wild-derived mouse strains. Extensive variation in the period and amplitude of the circadian rhythms suggest the clock is dynamic and genetically heterogeneous.

Powerful tools for investigating the genetics underlying the relationships between circadian rhythms and addiction-related behaviors are the Collaborative Cross (CC) and Diversity Outbred (DO) mouse populations. These populations are derived from an eight-way cross of five inbred and three wild-derived founder strains. The recombination of these genomes captures more than 45 million unique polymorphisms and variants, along with millions of novel allelic combinations, driving expansive genetic and phenotypic heterogeneity. As part of the Center for Systems Neurogenetics of Addiction, our goal is to use these mouse populations to identify and further understand the genetics of circadian rhythm and addiction-related phenotypes. We have begun to determine the heritability of molecular rhythm and cocaine intravenous self-administration (IVSA) phenotypes in the eight founder strains, and their potential genetic correlations in an effort to elucidate molecular drivers in reward-related brain regions contributing to drug-seeking.

**Methods:** Experiments were conducted in adult male and female mice of the founder strains (common inbred: A/J, C57BL/6 J, 129S1/SvlmJ, NOD/ShiLtJ, and NZO/HiLtj, and wild-derived: CAST/EiJ, PWK/PhJ, and WSB/EiJ). To measure molecular rhythms, ear punches were collected from naive mice of the eight founder strains ($n = 6$-10 mice per strain per sex) to derive skin fibroblasts to be transfected with lentivirus expressing luciferase fused to the circadian gene promoter of Bmal1 (Bmal1-dLuc). Fibroblast bioluminescence rhythms were measured for 5-7 days to assess period, amplitude, phase, and damping rate. To measure addiction-related behaviors, male and female mice of the eight founder strains ($n = 8$-12 per strain per sex) underwent cocaine IVSA behavior to assess acquisition and maintenance (seeking), dose-response (sensitivity), extinction and cue-induced reinstatement (relapse). Rhythm and drug phenotypes were compared between founder strains and sexes and heritability was calculated using between strain intraclass correlations.

**Results:** There was a substantial variability of molecular rhythm and cocaine IVSA phenotypes between founders. Heritability estimates for phase (38%), damping rate (57%), period (65%), and amplitude (91%), suggest significant genetic contribution to circadian phenotypes. Relative to C57BL/6 J mice, rhythm period were significantly shorter for 129S1/SvlmJ, WSB/EiJ, and CAST/EiJ female mice and significantly longer for A/J and PWK/PhJ male mice ($p < 0.05$). Rhythm amplitude was significantly higher for WSB/EiJ males and females (~3-fold) and 129S1/SvlmJ (~4-fold) compared to C57BL/6 J ($p < 0.05$). High heritability estimates were also found for acquisition and maintenance, and dose-response during cocaine IVSA (e.g., fixed-ratio 1 acquisition dose of 1 mg/kg per infusion, 43%). Wild-derived CAST/EiJ and PWK/PhJ males displayed the highest number (~50 to 65 infusions) for cocaine (1 mg/kg) during IVSA relative to each of the other founders (~15 to 25 infusions). Preliminary correlations between rhythm and drug-related phenotypes for the founders suggests lower amplitudes and delayed phases are associated with propensity to self-administer cocaine, prolonged extinction, and pronounced reinstatement.

**Conclusions:** Using the founder strains of CC and DO mouse populations, we demonstrate high heritability of molecular rhythm and drug-related phenotypes. Our preliminary analyses reveal potentially strong relationships, where less robust molecular clocks are associated with increased cocaine-seeking, and

consistent with the human literature, delayed phases and longer periods, may be related to dependence and relapse. Ongoing efforts are using RNA-seq across the circadian cycle among the founders with extreme rhythm and drug phenotypes to investigate the impact of genetic variation on the diurnal transcriptome within reward-related brain regions. Other behaviors are being investigated among founders, CC, and DO, including impulsivity and novelty-seeking, phenotypes associated with addiction.

**Keywords:** Addiction, Circadian Rhythms, Mouse Genetics, Cocaine Self-Administration, Collaborative Cross and Diversity Outbred Mice

**Disclosure:** Nothing to disclose.

### T53. Dissecting the Genetics of Alcohol Consumption and Alcohol Misuse, and Overlap With Alcohol Dependence

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**Background:** Alcohol use disorders (AUD) are modestly heritable, with twin-studies demonstrating that approximately 50% of the variance is attributed to genetic factors. Genetic studies of AUD have identified genes that influence pharmacokinetic factors (e.g. ADH1B, ADH1C), but the difficulty to obtain carefully diagnosed cohorts of AUD has slowed further progress in the field of alcohol genetics and stimulated additional studies of non-clinical phenotypes.

**Methods:** We obtained quantitative measures using the Alcohol Use Disorder Identification Test (AUDIT) from two population-based cohorts of European ancestry: UK Biobank (UKB; N = 121,630) and 23andMe (N = 20,328) and performed a genome-wide association study (GWAS) meta-analysis. We also performed GWAS for AUDIT items 1-3, which focus on consumption (AUDIT-C), and for items 4-10, which focus on the problematic consequences of drinking (AUDIT-P).

**Results:** The GWAS meta-analysis of AUDIT total score identified 11 associated risk loci. Novel associations localized to genes including JCAD and SLC39A13; we also replicated previously identified signals in the genes ADH1B, ADH1C, KLB, and GCKR. The dimensions of AUDIT showed positive genetic correlations with alcohol consumption (rg = 0.78-0.96) and Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) alcohol dependence (rg = 0.33-0.64). AUDIT-P and AUDIT-C showed significantly different patterns of association across a number of traits, including psychiatric disorders. AUDIT-P was positively genetically correlated with schizophrenia (rg = 0.22, p = 1.2 × 10-10), major depressive disorder (rg = 0.26, p = 4.5 × 10-5), and ADHD (rg = 0.23, p = 1.2 × 10-5), whereas AUDIT-C was negatively genetically correlated with major depressive disorder (rg = -0.23, p = 3.2 × 10-3) and ADHD (rg = -0.10, p = 1.8 × 10-2). We also used the AUDIT data in the UKB to identify thresholds for dichotomizing AUDIT total score that optimize genetic correlations with DSM-IV alcohol dependence. Coding individuals with AUDIT total score of ≤ 4 as controls and ≥ 12 as cases produced a high genetic correlation with DSM-IV alcohol dependence (rg = 0.82, p = 3.9 × 10-6) while retaining most subjects.

**Conclusions:** We conclude that AUDIT scores ascertained in population-based cohorts can be used to explore the genetic basis of both alcohol consumption and AUD.

**Keywords:** Alcohol dependence, Alcohol Consumption, Alcohol Use Disorder, GWAS, Human Genetics

**Disclosure:** Nothing to disclose.

### T54. Δ9-Tetrahydrocannabinol Enhances Fronto-Striatal Resting State Functional Connectivity

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**Background:** Accumulating evidence indicates that the main psychoactive component of cannabis, Δ9-Tetrahydrocannabinol (THC), activates brain reward circuitry, especially the nucleus accumbens (NAcc) and medial prefrontal cortex (mPFC). Few studies have examined how THC impacts brain reward circuitry in humans. In this study, we examined if an acute dose of THC altered functional connectivity between the NAcc and the mPFC among healthy young adults. We hypothesized that individuals receiving THC (vs. placebo) would show greater functional connectivity between the NAcc and the mPFC.

**Methods:** Participants were randomized to receive THC (n = 24) or placebo (n = 22) in a double-blind, between-subject design. Participants completed self-report measures of euphoria and drug-liking at regular intervals throughout the visit. Approximately 120 minutes after drug administration, participants completed an 8-minute resting state functional MRI (fMRI) scan. We utilized seed-based connectivity of the right and left NAcc to the whole brain. Group differences in self-report measures were examined using analysis of variance (ANOVA).

**Results:** THC increased functional connectivity between the right NAcc and the right mPFC, the right dorsomedial prefrontal cortex, and the right angular gyrus compared to placebo (p-values < .05, corrected). THC increased functional connectivity between the left NAcc and the left lingual gyrus (p < .05, corrected). THC also increased subjective euphoria ratings compared to placebo (p = .03). There were no significant group differences in subjective drug liking. Right NAcc-mPFC connectivity was not significantly related to euphoria ratings (p > .05).

**Conclusions:** This is one of the first studies to examine how THC alters resting state functional connectivity in healthy young adults. We found that THC increases resting state connectivity between the NAcc and the mPFC, regions implicated in reward, compared to placebo. The relationship between neural responses to THC and subjective effects to THC remains unclear. It is possible that behavioral indices of reward may be more closely related to neural response than self-report measures. Overall, our findings suggest that THC produces subjective and neural reward responses that may contribute to the rewarding, reinforcing properties of cannabis.

**Keywords:** Resting State Functional Connectivity, THC, Cannabis, Reward, Functional MRI (fMRI)

**Disclosure:** Nothing to disclose.

### T55. "Oops…I Did It Again": Relapse to Drinking During Outpatient Alcohol Treatment is Related to Elevated Brain Reactivity to Cues

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**Background:** Alcohol use disorder (AUD) is associated with elevated brain reactivity to alcohol cues in regions involved in reward processing and attention. Our lab previously demonstrated that a single session of continuous theta burst stimulation (cTBS) to the ventromedial prefrontal cortex (VMPFC) can reduce alcohol cue reactivity in heavy drinkers. The purpose of this double-blinded, active sham-controlled clinical trial was to assess the effects of 10 days of VMPFC cTBS on alcohol cue reactivity and relate this to treatment outcome in a cohort of treatment-engaged individuals with AUD.

**Methods:** Thirty individuals with AUD were randomized to receive 10 daily sessions of active or sham VMPFC cTBS (FP1 location, 3600 pulses, 110% rMT) while they were enrolled in an intensive outpatient treatment program and receiving behavioral treatment as usual. Brain reactivity to alcohol cues was assessed using a validated alcohol cue reactivity task before and after the 10 days of cTBS. Abstinence during treatment was determined by urinalysis (i.e. urine ETG levels). To identify neural patterns associated with abstinence, changes in alcohol cue reactivity were compared between individuals who maintained sobriety and those who used during treatment.

**Results:** Of the 30 participants, 16 abstained from alcohol use during the course of treatment. Baseline alcohol use (i.e. past month # drinking days) and alcohol cue reactivity did not differ between those who abstained and those who used. However, individuals who abstained from alcohol during treatment showed significant reductions in alcohol cue reactivity in the VMPFC and left frontoparietal network (i.e. left dorsolateral PFC and left superior parietal lobule) relative to individuals who used (cluster-level $pFWE < .05$, $k > 1326$). Attenuations were greatest in abstinent individuals who received active cTBS treatment.

**Conclusions:** These data suggest that reducing alcohol cue reactivity in the VMPFC and frontoparietal is associated with maintaining sobriety in AUD patients receiving concurrent TMS and outpatient treatment. These regions are known to be involved in cue reactivity and attention, and therefore these findings corroborate prior evidence indicating their importance in AUD and support these regions as neural biomarkers of potential for treatment success.

**Keywords:** Functional MRI (fMRI), Cue Reactivity, TMS, Alcohol, Relapse Biomarkers

**Disclosure:** Nothing to disclose.

### T56. Differential Associations Between Cortical GABA and Glutamate and Resting-State Functional Connectivity of the Dorsal Anterior Cingulate Cortex in Alcohol Use Disorder

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**Background:** Abnormalities in resting-state functional connectivity (rsFC) of the prefrontal cortex (PFC) have been reported among individuals with Alcohol Use Disorder (AUD), relative to healthy controls (HC). The neurochemical underpinnings of these abnormalities are unclear. GABA and glutamate, the primary inhibitory and excitatory neurotransmitters in the PFC, underlie many aspects of rsFC. Abnormal GABA and glutamate concentrations have also been reported in AUD, but no extant studies have examined the relationship between cortical concentrations of these neurotransmitters and rsFC in AUD. Dysregulation of cortical rsFC, GABA and glutamate have also been associated with impulsivity, a core feature of AUD. This study used resting-state functional magnetic resonance imaging (rsfMRI) and proton magnetic resonance spectroscopy (1H-MRS) to assess relationships between GABA and glutamate concentrations in the dorsal anterior cingulate cortex (dACC) and rsFC between dACC and the rest of the brain among individuals with AUD and demographically matched HC. For brain areas whose dACC-seeded rsFC was differentially associated with GABA/glutamate between groups, rsFC with dACC was subsequently analyzed for correlation with trait impulsivity.

**Methods:** Participants were 19 actively drinking young adults with AUD (mean age = 26.7 [SD = 5.1], 5 females, mean drinks per day = 6.1 [SD = 2.5]) and 19 HC (mean age = 24.6 [SD = 3.1], 6 females, mean drinks per day = 0.6 [SD = 0.5]). All participants had a negative urine drug screen and no measurable breath alcohol concentration prior to scanning. Trait impulsivity was measured with the UPPS-P Impulsive Behavior Scale, which has five subscales: Negative Urgency, Positive Urgency, Lack of Premeditation, Lack of Perseverance, and Sensation-Seeking. A 6-minute resting echoplanar imaging (EPI) sequence and a two-dimensional j-resolved (2D-J-PRESS) 1H-MRS sequence were acquired on a Siemens 3 T Trio scanner. The 1H-MRS acquisition voxel was a $25 \times 25 \times 30$ mm volume encompassing the dACC. After preprocessing the resting EPI images with FSL v. 5.0.9, correlations between the BOLD timecourse of a 6-mm-radius sphere centered on the dACC within the MRS acquisition voxel and every other voxel in the brain were calculated. 1H-MRS data were processed with the ProFit algorithm and 2D basis sets, and estimated GABA and glutamate metabolite peaks were ratioed to water and corrected for within-voxel CSF fraction. dACC-seeded rsFC maps, GABA and glutamate concentrations, and UPPS-P scores were compared between groups (AUD vs. HC). Using FSL FEAT v. 6.0, interactions between group and dACC GABA and glutamate were tested to identify, on a whole-brain basis, brain areas whose rsFC with dACC was differentially associated with dACC GABA and glutamate. Whole-brain analyses were thresholded using clusters determined by $z > 2.6$ and a cluster significance threshold of $p < .05$, corrected for multiple comparisons.

**Results:** AUD individuals, relative to HC, had significantly decreased rsFC between dACC and a variety of brain areas, including right medial and dorsolateral PFC, right postcentral gyrus, bilateral superior temporal gyrus, and left lingual gyrus. There were no areas in which AUD individuals had greater dACC-seeded rsFC than HC. AUD individuals, relative to HC, also had significantly lower GABA ($p = .017$), but not glutamate ($p = .89$), concentrations, and significantly higher scores on the UPPS-P Negative Urgency ($p < .001$) and Positive Urgency subscales ($p < .001$). Group differences in relationships between dACC-seeded rsFC and GABA and glutamate concentrations were identified in several brain areas. Specifically, the correlation between GABA concentrations and dACC-thalamus rsFC and dACC-cerebellum rsFC was positive among HC, but negative among AUD individuals. Similarly, the correlation between glutamate concentrations and dACC-inferior frontal gyrus (IFG) rsFC was positive among HC, but negative among AUD individuals. Across all participants, weaker (more negative) dACC-IFG rsFC was associated with higher scores on the UPPS-P Negative Urgency ($r = -0.39$, $p = .02$) and Positive Urgency ($r = -0.31$, $p = .057$) subscales.

**Conclusions:** These data replicate previous findings of abnormal cortical rsFC and GABA among individuals with AUD and indicate that these individuals exhibit strikingly different relationships than HC between cortical rsFC and cortical GABA and glutamate concentrations. In contrast to HC, among whom GABA and glutamate concentrations were positively correlated with dACC rsFC with thalamus, cerebellum, and IFG, AUD individuals exhibited diametrically opposite patterns of correlation. Acute and chronic alcohol effects on GABA and glutamate concentrations in AUD may change the nature of cortical networks supported by these neurotransmitters, rendering them less cohesive, and, in the case of connectivity between dACC and IFG (two regions long

implicated in cognitive control), more susceptible to impulsive urges. These findings suggest that interventions that normalize GABA concentrations and/or cortical network connectivity may merit further study for AUD treatment, particularly for more impulsive individuals.

**Keywords:** Resting State Functional Connectivity, Proton Magnetic Resonance Spectroscopy, Anterior Cingulate Cortex (ACC), Alcohol Use Disorder, Impulsivity

**Disclosure:** Laboratorio Farmaceutico CT, Grant

### T57. Neural Processing of Social Interactions Distinguishes Binge and Non-Binge Drinkers: A Machine Learning Approach

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**Background:** Neural processing differences in multiple brain regions have been found between heavy alcohol drinking individuals and comparison samples. Yet few studies have directly compared multiple tasks simultaneously to determine which best distinguishes regular binge drinkers from non-binge drinkers, where a binge is defined as more than 5 drinks a day. Identifying tasks that accurately discriminate binge drinkers from controls could help develop neuroimage biomarkers to predict clinical outcomes such as the development of an alcohol use disorder.

**Methods:** The Human Connectome Project database was searched to identify 117 young adults who binged at least once per week on average in the previous year. A comparison group of 117 adults who did not binge at all in the past year was matched to the binge drinkers based on age, sex, socioeconomic status, and body-mass index. 75% of the sample (88 binge, 88 non-binge) was used as a training sample and the rest of the sample was used as a holdout dataset. Data from each of the seven tasks implemented in the study (emotion processing, working memory, motor, social, gambling, relational, and language) were examined independently. For each task, blood-oxygen-level dependent (BOLD) signals were extracted from each of 378 brain regions derived from a multi-modal parcellation (anatomy, functional MRI, and resting state connectivity). The 378 variables were then processed through three separate machine-learning algorithms (support vector machine, random forest, elastic net) using a range of parameters to identify the best performing model on the training dataset via 10-fold cross-validation. Model performance was assessed by the area under the receiver operating characteristic curve (AUROC) to maximize both specificity and sensitivity. The best performing model was then tested on the holdout dataset. Behavioral measures were examined and compared to the machine learning models.

**Results:** The task that produced the largest area under the curve in the training set was the social processing task (AUROC = 0.65, SD = 12) using the support vector machine model. When this model was applied to the holdout set, it performed significantly better than chance at classifying binge drinkers and non-binge drinkers (AUROC = 0.72, $p < 0.05$). As the social task investigates neural processing related to theory of mind, we examined metrics of extraversion and friendship satisfaction and found that binge drinkers scored significantly higher than non-binge drinkers on both. Individuals with a higher probability of being a binge-drinker, as determined by the support vector machine model, had significantly higher scores on the friendship metric ($r = 0.21$, $p = 0.001$).

**Conclusions:** Relative to the other six tasks in this analysis, neural activity during a task that assesses social processing and theory of mind was associated with unsurpassed ability to distinguish binge drinkers from non-binge drinkers using machine learning algorithms. Binge drinkers showed greater levels of two social metrics, friendship satisfaction and extraversion, and these scores were related to the neural patterns identified in the machine learning algorithms. These results suggest that binge drinkers have differing traits and perceptions associated with socializing that manifest in altered neural processing of social-related mental tasks.

**Keywords:** Support Vector Machine (SVM), Alcohol, Social Behavior

**Disclosure:** Nothing to disclose.

### T58. Differences in Cognitive Effects of Intranasal Nicotine Administration and Subsequent Nicotine Reinforcement Among Young Adult Non-Smokers With and Without ADHD

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**Background:** Individuals with Attention Deficit Hyperactivity Disorder (ADHD) are at risk for a range of adverse smoking outcomes compared to the general population, including overall risk of use, earlier initiation, faster transition to dependence, and poorer cessation outcomes. However, little is known about the mechanisms underlying this risk. Smoking may be more reinforcing for those with ADHD due to nicotine's cognitive enhancing effects, which may mitigate underlying cognitive deficits (i.e., self-medication). Prior studies have demonstrated greater withdrawal-induced deficits in inhibitory control among ADHD smokers relative to controls, which were associated with greater smoking-reinforced responding. These findings support the role of cognitive function in maintaining smoking behavior among dependent smokers with ADHD. However, no experimental studies have been conducted to examine smoking risk among those with ADHD who are not established cigarette smokers, and thus it is unknown whether ADHD confers greater risk for initial smoking reinforcement via cognitive effects of nicotine. To address this question, we used a novel laboratory-based model of intranasal nicotine administration to examine changes in both subjective and objective measures of attention in response to two doses of nicotine, and nicotine reinforcement under high and low cognitive demand conditions, among non-smoking young adults (ages 18-25) with and without ADHD.

**Methods:** Participants with verified ADHD ($n = 60$) and healthy controls ($n = 75$) completed a series of 5 double-blinded nicotine administration sessions – 3 in which fixed doses of intranasal nicotine (0.0 mg, 0.5 mg, 1.0 mg) were administered (FD sessions), and 2 in which participants chose to self-administer nicotine or placebo under conditions of either high or low cognitive demand (CS sessions). Procedures during each of the FD sessions were identical except for the dose of nicotine being evaluated. During each FD session, participants received 3 separate nasal spray administrations spaced 45 minutes apart and completed 5 repetitions (2 pre-drug and 3 post-drug) of an assessment battery including both objective (continuous performance task; CPT) and subjective (self-reported concentration and alertness) measures of cognitive function. During the CS sessions, participants were re-exposed to 0 and 1 mg nasal sprays and then made a series of forced choices between the two options. Participants selected two sprays every 45 minutes, across 5 repetitions. During the high demand session, participants completed a 10-minute mathematical vigilance task following each drug administration; during the low demand session, participants engaged in sedentary activities. Repeated measures ANOVA was used to examine a) pre- to post-

drug changes in cognition during FD sessions as a function of nicotine dose and ADHD group; and b) total number of nicotine choices during CS sessions as a function of cognitive demand and ADHD group. In addition, pre- to post-drug changes in cognitive measures during the 1.0 mg FD session relative to the 0.0 FD session were examined as predictors of nicotine choices during CS sessions as a function of cognitive demand within each group.

**Results:** During FD sessions, main effects of group were seen for self-reported concentration and alertness, and for errors of commission on the CPT (all p's < .001), indicating greater deficits in these areas among ADHD relative to control participants. In response to nicotine, a group by dose interaction was seen for concentration ($F = 3.190$, $p < .05$); ADHD participants reported increased concentration following nicotine administration relative to placebo, whereas control participants reported decreased concentration for all doses. Nicotine increased errors of commission on the CPT relative to placebo, but this effect did not differ by group. During CS sessions, participants made more nicotine choices during the high relative to low demand session ($F = 8.564$, $p < .01$), and ADHD participants made significantly more nicotine choices than controls ($F = 3.928$, $p < .05$); however, the group by condition interaction was not significant. Within the ADHD group, nicotine-induced changes in errors of omission ($p < .05$) and commission ($p < .01$) on the CPT interacted with demand condition to predict choice behavior. Specifically, fewer errors of omission or commission following 1.0 mg nicotine administration was associated with greater nicotine choices during the high demand relative to low demand condition. Subjective improvement in concentration and alertness was unrelated to nicotine choices.

**Conclusions:** Consistent with considerable work showing differences in smoking related outcomes among regular smokers with and without ADHD, the present findings indicate that these differences extend to initial experiences with nicotine. Specifically, non-smokers with ADHD self-report greater increases in concentration following nicotine administration compared to controls. Moreover, non-smokers with ADHD self-administer nicotine more than controls, regardless of cognitive condition. Finally, results indicate that for ADHD non-smokers (but not for controls), nicotine-induced changes in objectively measured cognitive function predict nicotine self-administration under conditions of high relative to low cognitive demand. These results have significant implications for understanding the underlying risk for adverse smoking outcomes among those with ADHD.

**Keywords:** ADHD, Nicotine, Attention, Cognition

**Disclosure:** Nothing to disclose.

### T59. Partial mGlu5 Negative Allosteric Modulator M-5MPEP Attenuates Cocaine-Related Behaviors and Cocaine-Induced Increases in Cerebral Blood Volume in Rats

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**Background:** Despite decades of research there are still no FDA-approved medications for Cocaine Use Disorder (CUD). Cocaine acutely increases mesocorticolimbic circuit function and repeated drug-stimulus pairings lead to glutamate-mediated synaptic plasticity changes within this circuit that contribute to hyper-responsiveness to drug-related cues and contribute to relapse. Possible approaches to preventing relapse include attenuating excessive brain responses to drugs acutely as well as long lasting cue-induced increases. Receptor function and localization suggest that antagonism via negative allosteric modulation of the metabotropic glutamate receptor subtype 5 (mGlu5) is a promising potential treatment for CUD. mGlu5 negative allosteric modulators (NAMs) can attenuate cocaine self-administration (SA) and cue- or drug-induced increases in previously extinguished responses following SA (reinstatement model of relapse) in animals. However, full mGlu5 NAMs engender dose-limiting adverse effects. Recently, we reported the discovery and characterization of highly selective partial mGlu5 NAMs, represented by M-5MPEP, that block less than 100% of the effects assessed in vitro when compared to a full mGlu5 NAM, at concentrations that fully occupy the allosteric binding site on mGlu5. These partial mGlu5 NAMs produce antidepressant- and anxiolytic-like effects without the adverse effects observed with full mGlu5 NAMs. Here, we describe studies comparing behavioral effects of the partial mGlu5 NAM M-5MPEP in rodent models of CUD. Further, to better understand brain-behavior relationships, we examined the ability of M-5MPEP to reverse cocaine-induced elevations in cerebral blood volume (CBV; a surrogate of brain function) using functional magnetic resonance imaging (fMRI) in rats. Examining effects of mGlu5 NAMs on cocaine-maintained behaviors and cocaine-induced brain changes will provide valuable insight into the pharmacotherapeutic potential of partial mGlu5 NAMs for treating CUD.

**Methods:** Separate male Sprague-Dawley rats implanted with indwelling intravenous catheters were used for behavior ($n = 60$) or imaging studies ($n = 20$). Rats were trained to lever press to SA 0.5 mg/kg/infusion of cocaine under a 5-response, fixed ratio (FR5) schedule, during daily 2-h sessions followed by 10 days of 6-hr extended access SA. Each SA session was paired with a vanilla odor cue and each infusion was paired with a 10-sec light cue presentation. Following extended access SA, responding was extinguished during daily 2-hr sessions in which saline was substituted for cocaine, the odor cue was absent, and the light cue was not presented following completion of each ratio. Once responding was reduced by ≥ 80% compared to the first day of extinction training, the light and odor cues were reintroduced in a single cue-induced reinstatement (CIR) test. To examine the ability of the mGlu5 partial NAM to attenuate CIR, 18-56.6 mg/kg M-5MPEP or vehicle (10% Tween 80) was administered (i.p.) 30 min prior to a single reinstatement session. Following CIR, cocaine SA was continued under a progressive ratio (PR) schedule of reinforcement. Lastly, rats responded under the same PR schedule when sucrose pellets were available as the reinforcer. M-5MPEP was administered 30 minutes prior to cocaine or sucrose pellet SA sessions. In a separate cohort, following extended access SA, effects of M-5MPEP were evaluated on extinction learning by administering 56.6 mg/kg M-5MPEP daily 30 min prior, or immediately following the first 5 days of response extinction sessions.

For fMRI studies, rats were anesthetized and changes in CBV were examined following administration of 0.5 mg/kg cocaine, (i.v.), 56.6 mg/kg M-5MPEP, (i.p.), or a 40-minute pretreatment of M-5MPEP prior to cocaine administration. Contrast-enhanced CBV studies were conducted at 9.4 T (Varian) using a Doty-Litz 38 mm transmit-receive RF coil under anesthesia (0.9% isoflurane delivered in N2O:O2 2:1). Rats were intubated and mechanically ventilated; vital signs were continuously monitored. A fast spin-echo sequence captured changes in CBV across the whole brain (TR/TE = 2600 ms/36 ms, FOV $35 \times 35$ mm, 14 1-mm thick slices). Following motion correction, brain masking, and registration to a template (MATLAB and AFNI), regions of interest were propagated from the rat MRI template to all co-registered subjects. Changes in CBV time courses for each ROI and maps were then generated for each animal. For all studies, ANOVAs were used for statistical analysis to examine differences from vehicle-treated control groups, followed by post-hoc Bonferonni t-tests.

**Results:** In behavioral studies, M-5MPEP dose-dependently (32, 56.6 mg/kg; $p < 0.05$) attenuated cue-induced reinstatement at doses that did not significantly affect food-maintained responding

nor extinction learning. 56.6 mg/kg M-5MPEP attenuated low doses of cocaine SA under a PR schedule ($p < 0.05$), and attenuated cocaine-induced increases in CBV in regions implicated in reward-related processes.

**Conclusions:** These results indicate that partial negative allosteric modulation of the mGlu5 receptor may be sufficient to attenuate cocaine-related behaviors modeling multiple aspects of substance use disorder without adverse effects associated with full mGlu5 NAMs. Together, behavior and imaging data support further development and understanding of partial mGlu5 NAMs as a promising pharmacotherapy for the treatment of CUD.

**Keywords:** Cocaine Self-Administration and Reinstatement, Functional MRI (fMRI), Metabotropic Glutamate Receptor 5 (mglu5), Negative Allosteric Modulator, Behavioral Pharmacology

**Disclosure:** Nothing to disclose.

### T60. Manipulation of the Transcriptome in the Adult Medial Amygdala Recapitulates the Sex-Specific Effects of Adolescent Social Isolation Stress on Complex Behavior

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**Background:** Adolescence, a time of heightened sensitivity to rewarding stimuli, is associated with vulnerability to psychiatric disorders. Social isolation stress (SI) during adolescence, but not adulthood, causes permanent changes in reward-associated behaviors in males. However, little is known regarding how females respond to adolescent SI. Our preliminary data suggest that adolescent SI reverses sex differences in reward behaviors and permanently reduces baseline sex differences ($M > F$) in neuronal projections from medial amygdala (meAMY) to ventral tegmental area (VTA). Given these circuit-specific and behavioral alterations, we tested the hypothesis that SI alters the meAMY transcriptome in a persistent and sex-specific manner, resulting in long-term changes in sexually dimorphic behaviors.

**Methods:** Male and female mice were isolated or group housed (GH) from postnatal day P22 - P42, then GH until ~P90. Transcriptome-wide changes in meAMY were investigated by RNA-seq after cocaine (acute/chronic) or saline ($n = 6$-8/group). Gene co-expression network analysis was conducted and key drivers of sexually dimorphic gene expression were identified. To determine if manipulation of a key driver gene could recapitulate the effects of SI, two genes of interest were virally overexpressed in the adult meAMY using AAV2.

**Results:** Sexually dimorphic genes were disproportionately affected by SI (Sex X SI: 869 genes). Gene co-expression analysis revealed that SI results in the loss of sexually dimorphic gene co-expression in the meAMY and identified key drivers of sex-specific patterns of gene expression. Overexpression of two key drivers, Crystallin Mu (Crym) or Regulator of Calcineurin 2 (Rcan2), in the adult meAMY induced sex-specific effects on baseline sexually dimorphic and reward-associated behaviors. Specifically, Crym overexpression caused a loss of sexually dimorphic behaviors through male-specific effects, whereas Rcan2 reduced sex differences through female-specific effects.

**Conclusions:** These data suggest that the meAMY plays an important role in sex differences in cocaine reward and that SI disrupts sex-specific adolescent development. Overexpression of Crym or Rcan2 in the adult meAMY not only recapitulates the effects of SI but induces sex-specific behavioral plasticity in adult animals. Together, these data suggest that the meAMY is a key brain region in the regulation of sex differences in reward-associated behavior.

**Keywords:** Medial Amygdala, Sex Differences, Cocaine, Adolescent Stress, Gene Expression

**Disclosure:** Nothing to disclose.

### T61. Glutamatergic Ventral Pallidal Neurons Modulate Activity of the Habenula-Tegmental Circuitry and Constrain Reward Seeking

Abstract not included.

### T62. Manipulation of Central Amygdala Neurotensin Neurons Alters the Consumption of Rewarding Liquids

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**Background:** The central nucleus of the amygdala (CeA) is a heterogeneous structure that plays an important role in the regulation of appetitive, aversive, and ethanol-mediated behaviors. While some data have shed light on the neuronal subpopulations influencing fear- and feeding-related behaviors, it remains unclear which CeA efferents and neuronal subpopulations influence ethanol consumption. One CeA neuron subpopulation that may regulate ethanol phenotypes are the neurons that produce the 13 amino-acid neuropeptide neurotensin (NTS). Indeed, considerable evidence suggests that NTS systems are critical for reward and anxiety processes, and global manipulations of the NTS system disrupt ethanol related phenotypes. The majority of studies investigating NTS-expressing neurons, however, focus on those that project from the lateral hypothalamus (LH) to the ventral tegmental area (VTA) and on NTS interactions with dopamine.

Little is known about the role that CeA projections to the hindbrain play in the context of ethanol consumption. Early studies found that the CeA projection to the hindbrain's parabrachial nucleus (PBN) contains NTS + fibers. The PBN plays a role in the development of conditioned taste aversion, as well as in fluid satiation. Additionally, the PBN is activated by intraperitoneal injections of ethanol. The PBN, in turn, sends projections back to the CeA that suppress food consumption. In order to investigate the complex relationship between the CeA and PBN, and to better understand the role of the CeA-NTS neuronal subpopulation in ethanol consumption and appetitive behaviors, we utilized NTS-ires-cre mice in conjunction with region-directed genetic lesioning, optogenetic stimulation, and behavioral assays.

**Methods:** Mice All procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, as adopted by the NIH, and with approval of an Institutional Animal Care and Use Committee at UNC-Chapel Hill. Adult male ( > 22 g) NTS-ires-cre mice (Jackson Laboratories, Bar Harbor, ME) were used for all experiments. Animals were maintained on a reverse 12-hour light cycle.

Electrophysiology was performed as previously described in Pleil et al. 2015.

Ethanol drinking 2-bottle choice (3, 6, and 10%), intermittent access (20%, Hwa et al 2011), and 3-hour access Phenotyper drinking (6%, Noldus Information Technology, Netherlands)

Other behavioral assays All locomotor and anxiety assays were performed using the Ethovision XT tracking software (Noldus Information Technology, Netherlands) to measure location, distance moved, and velocity. Real-time place preference, open field, elevated plus maze, novelty-suppressed feeding, and marble burying were performed. Optical intracranial self-stimulation was performed using Med Associates. All behavioral assays were performed during the dark cycle.

Data analysis Data are presented as mean ± SEM. Data was first tested for normality using the D'Agostino-Pearson test. Where data was not normal, a Wilcoxon matched-pairs test was performed. If data was normal, a Student's t-test, paired t-test or matched 2-way ANOVA was performed where appropriate. If a significant interaction was detected in the 2-way ANOVA, a post hoc Bonferroni test was performed. All statistical analyses were performed using GraphPad Prism version 6.02 for Windows (GraphPad Software, La Jolla California USA).

**Results:** We injected a cre-dependent virus expressing a modified Caspase 3 and TEV protease into the CeA of NTS-cre mice, ablating on average 51.7% of the NTS-positive cells in the CeA. This ablation reduced ethanol consumption in our mice but did not affect total liquid intake or body weight.

We next injected the CeA of NTS-cre mice with a cre-dependent Channelrhodopsin-eYFP (ChR2-eYFP) virus. We found that these cells send a very strong projection to the PBN, where stimulation releases GABA. Optical stimulation of the NTSCeA → PBN pathway conferred a positive valence and served as a reinforcer. Furthermore, optical stimulation of this pathway enhanced the consumption of ethanol, sucrose, and saccharine (all rewarding or sweet liquids), but not water or a quinine solution (neutral or bitter liquids). Surprisingly, stimulation failed to induce consumption of normal chow, or a sweet food (Fruit Loops), in both sated and hungry animals.

Neither neuronal ablation (Caspase), or neuronal activation (ChR2-eYFP) altered anxiety like behaviors.

**Conclusions:** These data suggest that NTS-expressing CeA neurons, and especially their projection to the PBN, play a significant role in modulation of specific consumptive behaviors and may alter incentive salience.

**Keywords:** Central Nucleus of the Amygdala, Ethanol, Optogenetics, Parabrachial, Reward

**Disclosure:** Blackthorn Therapeutics, Grant (Spouse)

## T63. Drug-Context Associations in the Dorsal Striatum

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**Background:** Cocaine addiction involves the encoding and retrieval of drug-related experiences that shape future behavior. Drug-reward associations are a key driver of both initial reinforcement learning and relapse after prolonged drug exposure. Using genetic tagging strategies, we have previously found evidence that a population of neurons in the dorsal striatum that is activated by initial drug exposure is preferentially reactivated during conditioned place preference testing. Yet the specific coding and causal contribution of these reactivated neurons has not been studied.

**Methods:** To investigate the neural coding of drug-context associations, we performed calcium imaging using wireless miniature microscopes in the dorsal striatum during conditioned place preference (CPP) to cocaine. We trained mice (n = 4) on two separate CPP chambers, one paired with cocaine and saline on each side (Paired chamber), and the other paired only with saline on both sides (Unpaired chamber). We then compared the activation of neural ensembles between training and testing. In addition, to determine the causal contribution of training-activated neurons to CPP, we used a viral TetTag approach to tag activated neurons during initial cocaine exposure and inhibit activated cells with hM4Di during CPP test (n = 8 per group).

**Results:** Using calcium imaging, we found that neurons activated during the training sessions were more likely to be reactivated during the CPP test in the Paired chamber compared to the Unpaired chamber. In addition, using TetTag inhibition we found that inhibiting cells in the dorsal striatum activated by cocaine training was sufficient to eliminate CPP. Conversely, inhibiting neurons that were activated by saline training did not eliminate CPP as animals continued to have a preference for the cocaine-paired side.

**Conclusions:** These results indicate that the neural ensemble activated during initial drug exposure is reactivated during drug-context memory retrieval. Furthermore, these activated neural ensembles are critical for the decision to go to the drug-paired side. Thus, the dorsal striatum is a site of plasticity driving drug-context associations that contribute to cocaine CPP and is a strong candidate to be a useful target for future interventions to reduce cocaine addiction and relapse.

**Keywords:** In Vivo Calcium Imaging, Conditioned Place Preference, c-Fos-Expressing Ensembles

**Disclosure:** Nothing to disclose.

## T64. Regional Specificity and Coordination of Dopamine Dynamics in Different Functional Domains of the Striatum in the Context of Flexible and Inflexible Reward Seeking

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**Background:** The main input structure of the basal ganglia, the striatum, consists of several functional domains that are defined by their afferent projections. The ventromedial striatum (VMS) receives predominantly "limbic" input, the dorsomedial striatum (DMS) "associative" input, and the dorsolateral striatum (DLS) mainly sensorimotor input. The release of dopamine in the striatum plays a prominent role in the regulation of these domains and critically shapes motivation, movement, and reward learning. However, the precise information conveyed by striatal dopamine signals, their temporal stability, and their regional specificity and coordination are under active debate.

**Methods:** We further characterized dopamine release using chronically implantable microelectrodes for fast-scan cyclic voltammetry simultaneously in VMS, DMS, and DLS of rats performing in different reward-driven operant conditioning paradigms. This approach enabled us to investigate dopamine dynamics in real-time resolution across trials, daily sessions, and weeks of behavioral training throughout the development of inflexible, habitual behavior. Furthermore, we tracked inter-regional coordination of trial-by-trial dopamine release.

**Results:** Our findings demonstrate that distinct dopamine signals are delivered to different functional domains of the striatum. VMS dopamine is consistent with a reward-prediction error (RPE) signal that is not affected by training duration, whereas DMS and DLS dopamine signals were less RPE-related and underwent changes across behavioral training. Furthermore, we found an involvement of dopamine in both motivation and learning and differential temporal emergence of regional signals depending on the type of conditioning paradigm. Trial-by-trial

coordination between VMS and DLS changed across behavioral training and differed between natural and drug rewards.

**Conclusions:** Although we found that all of the sampled striatal domains exhibited reward-related dopamine release, substantial heterogeneity existed across different functional domains with regards to signal make-up and the information encoded by the signal. Moreover, regional signals correlated only weakly with one another with little exception and displayed differences in long-term temporal stability. Together, these findings demand careful future investigation of such heterogeneous dopamine release dynamics and their coordination.

**Keywords:** Dopamine, Striatum, Reward Learning, Habit Formation

**Disclosure:** Nothing to disclose.

### T65. Exploring Epigenetic Regulation of BDNF and Other Genes in Patients With the Obsessive Compulsive Disorder

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**Background:** Obsessive Compulsive Disorder (OCD) is a psychiatric disorder characterized by obsessions (intrusive distressing thoughts) and/or compulsions (repetitive mental or behavioral acts). OCD affects about 2% of the world's population and it represents a high disabling condition with poor treatment outcome. Focusing on OCD etiology, there is a good evidence of genetic contribution, although environmental factors are also known to play an important role. Genetic studies have identified genes and relative products potentially involved in the pathophysiology of OCD, such as the brain derived neurotrophic factor (BDNF) and different monoamine receptor systems. Several candidate genes have been investigated, although the results are still sparse due most likely gene-environment interactions. Epigenetics, therefore, is supposed to play a key role in these interactions. The aim of the present study was to generate preliminary evidence about how these mechanisms can contribute to individual OCD development.

**Methods:** The study sample consisted of 24 OCD subjects on stable pharmacological treatment and 22 healthy controls. Diagnoses were assessed by the administration of semi-structured interviews based on DSM-5 criteria (SCID I and II, research version). Control subjects were volunteers matched for gender, age and ethnicity, without any psychiatric diagnosis, as determined by the SCID-I. Blood samples were collected and total DNA and RNA were isolated from peripheral blood mononuclear cells (PBMCs). PBMCs, in fact, are accessible cells with potential for biomarker discovery in psychiatric disorders, containing the full complement of epigenetic enzymes found in most tissues, including neurons. To assess target genes abundances, we used Real-Time RT-PCR and a non parametric Mann-Whitney test (significance level > 0.05) for statistical analyses. Moreover, in order to quantify gene promoters DNA methylation, we used pyrosequencing and multiple t test (Sidak-Bonferroni method; significance level > 0.05).

**Results:** Statistical analyses showed an up-regulation of BDNF, membrane-bound Catechol-O-methyltransferase (MB-COMT) and dopamine transporter (SLC6A3) in OCD subjects compared to controls, whereas DNA methylation levels resulted to be consistently reduced just at BDNF promoter. A down-regulation of serotonin transporter (SLC6A4), oxytocin receptor (OXTr) and monoamine oxidase B (MAOb) was also observed, without changes in DNA methylation levels.

**Conclusions:** Reported differences in up- and down-regulations of specific genes potentially involved in the pathophysiology of OCD along with a reduced methylation level at BDNF promoter represent a first attempt to understand the nature of genetic and epigenetic (gene x environment) interactions in patients suffering from OCD. A better understanding of gene-environment interactions as well as genetic pathways implicated in OCD genesis represents a major challenge in the field, in order to gain knowledge on OCD pathophysiology, develop new preventive and therapeutic strategies through specific modulation of epigenetic factors.

**Keywords:** Obsessive Compulsive Disorder, Epigenetics, Monoamines, BDNF, Pathophysiology

**Disclosure:** Livanova, Inc., Angelini and Lundbeck, Grant, Angelini, FB Health and Lundbeck, Honoraria

### T66. The Relationship Between COMT Genotype, Estradiol, and Episodic Memory in Post Menopausal Women

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**Background:** The prior literature on menopause and cognition has found large individual differences in whether or not women experience cognitive changes. In an effort to begin to disentangle the individual differences in cognition after menopause, the current study examined how a gene related to functioning of the dopaminergic system, catechol-O-methyltransferase (COMT) and estradiol were related to brain functioning in healthy postmenopausal women. The dopaminergic system may be important for cognition after menopause because age-related changes in the dopaminergic system have been implicated in normal cognitive aging (Braver & Barch 2002) and COMT and estradiol has been shown to influence cognition in pre-menopausal women (Jacobs & D'Esposito 2011).

**Methods:** Participants were 118 healthy, cognitively normal postmenopausal women between the ages of 50-60. All women provided a blood sample for COMT and estradiol analyses and underwent an MRI scan on a 3 Tesla scanner. Brain activation during episodic memory encoding was measured with BOLD fMRI during a face-name encoding task.

**Results:** Results were examined across each COMT genotype and a median split was performed on the circulating estradiol levels to create high and low estradiol groups for each genotype. COMT genotype and estradiol level were hypothesized to be proxy measures for brain dopamine levels with the Met/Met and high estradiol group having the most dopamine and Val/Val and low estradiol group having the least dopamine. The fMRI results showed that the face-name encoding task activated the episodic encoding network including bilateral hippocampus, fusiform face areas, and left prefrontal areas as well as deactivation in the bilateral anterior cingulate and posterior parietal cortices. However, no main effects of COMT genotype or estradiol group were found. There was COMT-estradiol interaction found in a small area of decreased activation in the right precuneus (Brodmann Area 7) that was related to the increasing hypothesized dopamine level. Specifically, women with a Met/Met genotype in the high estradiol group had the least activation in this frontal lobe working memory region. Women with a Val/Val genotype in the low estradiol group had greater activation in this region relative to the other groups. Recognition memory performance after the MRI session for the

face name combinations did not did not show any group differences.

**Conclusions:** These data indicate that after menopause COMT genotype and potentially the menopause-related changes to the dopaminergic system are not related to episodic memory encoding. Future studies should examine how the relationship between COMT, estradiol, and cognition around the menopause transition as there appear to be differences in this relationship for pre and postmenopausal women.

**Keywords:** Menopause, COMT Gene, Estradiol, fMRI, Epiodic Memory

**Disclosure:** Nothing to disclose.

### T67. Resting State Brain Network Coherence and Cognition During Late Life Depression and Healthy Aging

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**Background:** There has been a growing interest in the neuroimaging literature into the functioning and coherence of brain networks during the resting state, in contrast to single regions of interest. Brain networks relevant to cognition include the Default Mode Network (DMN) and Cognitive Control Network (CCN). There is some evidence that connectivity within these networks predicts performance variations among individuals with psychiatric and neurologic diseases, though less is known about how this compares to such relationships in healthy aging. This study sought to elucidate relationships between resting state network coherence of the DMN and CCN and performance on a comprehensive battery of neuropsychological tests in late life depression and healthy aging.

**Methods:** Thirty-one never-depressed, cognitively normal older adults (HC; M age = 70, SD = 6) and 13 older adults with Major Depressive Disorder (MDD; M age = 66, SD = 4) completed a comprehensive neuropsychological test battery and an 8-minute resting state fMRI scan. Independent component analysis (ICA) was used to segregate DMN and right and left CCN for each participant using the GIFT Toolbox. RS networks were identified using a fully automated template matching procedure using previously published templates (Smith et al., 2009). Using network masks obtained by thresholding the same previously published templates at $Z > 3$, Z-scores representing average connectivity strength within each entire network were extracted for each participant. ANCOVAs were conducted to examine between-group differences in network strength, covarying for age, site, and individual correlation values with the template brain. A series of multiple regression analyses were conducted with DMN, left, or right CCN as the predictor variable, covarying for age, scanner, and cross correlation values with the template brain. Age-corrected neuropsychological test scores composed outcome variables.

**Results:** HC and MDD did not significantly differ on performance on neuropsychological measures. HC had greater network coherence in left CCN, F (1, 39) = 4.84, p = .03, partial eta2 = .11 and DMN, F (1, 39) = 5.26, p = .03, partial eta2 = ..12. Left CCN coherence positively predicted performance on measures of concept formation and verbal fluency (Adj. R2s = .22 - .45, all ps < .05). In HC, DMN connectivity positively predicted performance in a variety of cognitive domains, including memory retrieval, working memory, and executive functioning (Adj. R2s = .22-.41, all ps < .05). Connectivity values were not significantly predictive of cognitive performance on any measure in MDD.

**Conclusions:** Despite similar cognitive performances, older adults with MDD demonstrate less within-network coherence in left CCN and DMN, relative to their never-depressed peers. Moreover, resting state functional brain network coherence is predictive of cognitive performance in HC, but not in MDD. Small sample size in MDD may have contributed to this lack of association. Extent of coherence during RS in specific brain networks may be predictive of future cognitive decline. Ongoing data collection follows this sample longitudinally, with annual cognitive assessments.

**Keywords:** Resting State Functional Connectivity, Older Adults, Depression, Cognition

**Disclosure:** Nothing to disclose.

### T68. Visualizing the Cholinergic System in Health & Disease

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**Background:** Our laboratory seeks to determine the role of the cholinergic system in memory and cognition. Cholinergic neurons of the basal forebrain send extensive projections to the cortical mantle and many subcortical regions of the brain including the hippocampus and the amygdala (Zaborszky et al. 2012). The broad reach of these cholinergic projections, coupled with the varied timescales over which ACh is released (Sarter et al. 2015), grants cholinergic neurons highly flexible, context-specific control over cortical dynamics (Zaborszky et al. 2015).

Recent advances in molecular genetic techniques permit targeted and detailed dissection of the cholinergic system. Such studies highlight the importance of functionally organized cholinergic terminal fields in both rapid and prolonged control of network function. Sparse labeling studies reveal that individual cholinergic neurons from mouse basal forebrain have complex, extensively branched axonal arbors covering up to 30 cm of length (in a brain that is 2 cm long!) (Wu et al. 2014). These highly branched axonal arbors may be metabolically challenging to maintain, perhaps underlying the vulnerability of cholinergic axons to fragmentation and loss in the aging brain. Indeed, studies of post-mortem brains from patients with severe AD find significant loss of cholinergic fibers compared to their age-matched, cognitively intact counterparts (Geula et al. 1996). Given these findings, we have focused on developing methods to map the complex architecture of cholinergic axonal arbors in normal and pathological aging.

Aligned with the work of the BRAIN initiative, we take advantage of recent technological advances to perform high-resolution mapping of cholinergic circuits. Coupled with genetically targeted fluorescent probes, we can reconstruct whole brain cholinergic circuits in rodents. Because this high-resolution investigation is not possible in humans, we use a synaptic marker of cholinergic neurons, Positron Emission Tomography (PET) tracer 18 F VAT, as a surrogate measure of whole-brain cholinergic activity in vivo. We then use information gathered from the high-resolution imaging in rodents to inform our studies probing the mechanisms underlying alterations in VAT binding in healthy vs. cognitively impaired humans.

**Methods:** All studies were conducted in accordance with Stony Brook University's Institutional Animal Care and Use Committee (IACUC) and Institutional Review Board (IRB). To investigate the cholinergic system in rodents, we use a transgenic mouse model specifically designed for visualization and quantification of all cholinergic cell bodies and, more importantly, all aspects of the

cholinergic projection fields by way of a cytoskeleton-associated green fluorescent protein (ChAT tau-eGFP) (Grybko et al. 2011). By crossing the ChAT tau-eGFP mouse to an aging model (mouse model that exhibits accelerated aging pathology including amyloid plaques and hyperphosphorylated tau tangles), we are able to investigate changes to cholinergic projection profiles in aging. Using specific cognitive tasks that assay cortical function, we can stratify the data on cholinergic system integrity by performance in cognitive tasks. In parallel in vivo studies in humans, we acquired18F VAT scans in healthy patients and patients with cognitive impairment to visualize and quantify changes in the pattern and density of cholinergic innervation in health vs disease.

**Results:** Using wide-field optics microscopic analyses of normal ChAT-eGFP vs aging model mice, we are assembling maps of the cholinergic axonal arbors and cell bodies throughout the anterior-posterior extent of the mouse brain. Our collaborators developed data extraction and registration algorithms that achieve confocal-like resolution from the wide-field optics microscope, greatly improving processing time (Boor Boor et al. 2018). Our initial studies in aging model mice demonstrate dramatic fragmentation of cholinergic axonal fibers in the entorhinal cortex (EC; a region vulnerable early on in aging (Khan et al. 2014)). In addition, we find changes in the number of cholinergic neurons in cholinergic nuclei that project to the EC. We have begun to analyze these data in a high-resolution, immersive gigapixel facility (one of the few in the world) (Papadopoulos et al. 2016), to determine the relationship between cholinergic terminal field density and EC function (assessed using an EC specific behavioral task). In ongoing parallel human studies, we use 18F VAT for in vivo quantification of cholinergic synaptic density. Preliminary findings suggest a correlation between changes in cholinergic terminal field density and cognitive impairment in humans.

**Conclusions:** The strength of these studies lies in our ability to apply high-resolution techniques in both rodents and humans to better understand the organization of the cholinergic system. What we lack in resolution in human PET experiments, we accomplish with detailed imaging of the genetically labeled cholinergic connectome in mice; what we lack with direct disease applicability in the mouse, we can accomplish in the human studies. Using related markers of cholinergic terminal fields, albeit with dissimilar techniques, has strengthened our conviction that the highly quantitative information that can be gathered in rodents will improve our interpretation of in vivo human imaging and allow us to make specific predictions about what altered VAT binding tells us about cognition and cognitive impairment.

**Keywords:** Cholinergic System, Cognitive Impairment, Whole-Brain Rodent Imaging, PET Imaging, Translational Neuroscience

**Disclosure:** Nothing to disclose.

### T69. Functional Connectivity Between Networks Predicts Acute Outcome in Geriatric Depression

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**Background:** Functional magnetic resonance imaging (fMRI) studies in younger depressed adults have reported associations between increased functional connectivity in frontal and limbic brain regions and response to antidepressant medications. Given the scarcity of fMRI studies of antidepressant response in the elderly, we similarly hypothesized that functional connectivity within and between frontal and limbic regions would be associated with 12-week depression outcomes in older depressed adults.

**Methods:** 71 older depressed adults were enrolled in the Neurobiology of Late-Life Depression (NBOLD) study at the University of Connecticut School of Medicine. All patients met criteria for major depression and were free of dementia at baseline. Upon enrollment in the study, all subjects were interviewed by a study geriatric psychiatrist, who also administered the Montgomery-Asberg Depression Rating Scale (MADRS) and confirmed a diagnosis of major depression. All subjects were scanned on a Skyra 3 T scanner (Siemens, Erlangen, Germany) with 32 surface coils located at the Olin Neuropsychiatry Research Center at the Institute of Living of Hartford Hospital. Resting-state functional images were acquired using simultaneous multislice echo-planar imaging (EPI) sequence (factor = 8) with TR/TE = 475/30 ms, flip angle = 60o, matrix = $80 \times 80 \times 49$, and voxelsize = $3 \times 3 \times 3$ mm. Resting-state fMRI data were preprocessed using the default settings of functional connectivity (CONN) toolbox v17a. (23) including realignment, segmentation, coregistration, normalization, smoothness using a Gaussian kernel of 8 mm3, and band-pass filtering (0.008 ~0.01 Hz). Further denoising steps included regression of the six motion parameters and their first-order derivatives, and regression of white matter and cerebrospinal fluid (CSF) signals. Using the regions of interests (ROI) atlas provided by CONN toolbox (23), we computed ROI-to-ROI functional connectivity (FC) for each participant. All subjects were offered initial treatment with sertraline, with dosing titration every two weeks, depending on clinical status and toleration of medication. Patients were followed every two weeks by a study geriatric psychiatrist. Those that could not tolerate sertraline or who had persistent depression were offered either augmentation with bupropion or switch to desvenlafaxine. The study psychiatrist completed a MADRS at each visit through 12 weeks. In the Statistical approach, multivariate regression analysis was used to examine correlation between change of MADRS score over 12 weeks and functional connectivity between brain regions, controlling for age, gender, mean head motion, and baseline MADRS. The main effect of each ROI seed with all other ROIs was examined. A post-hoc test on each ROI-to-ROI functional connectivity correlation with MADRS changing score was also tested. Significant level was set at $p < 0.05$ with False Discovery Rate (FDR) correction. A permutation test with 1000 iterations was also used to confirm the results.

**Results:** 71 older depressed subjects were enrolled in the study. The group had a mean age of $71 + 6.60$ years, was 66.2% female, had a mean educational level of $20 + 5.81$, and had mean baseline MADRS score of $20 + 5.81$ and MMSE score of $29 + 1.30$.

In terms of treatment, 52 depressed subjects initiated treatment with sertraline, 12 with desvenlafaxine, one on paroxetine, one on escitalopram, one on a combination of escitalopram and bupropion. Four were started on other medications by their private psychiatrist. We calculated change in MADRS score from baseline to 12 weeks for each subject. The mean MADRS score at 12 weeks was $9.73 + 6.48$. In analyses controlling for age, gender, and baseline depression severity, we did not find a significant main effect of correlation between MADRS and functional connectivity between any ROI seed and all rest of ROIs. However, we found several pairs of regions whose baseline functional connectivity was associated with change in MADRS score.

**Conclusions:** The anterior cingulate cortex frontal eye fields are related to attention. The pars triangularis of the inferior frontal gyrus is involved in language processing and cognitive inhibition (including emotion regulation). High connectivity between IFG-FEF and between these regions with motivation (insula), reward (caudate, nucleus accumbens) and sensorimotor areas at baseline were associated with greater 12-week decrease in depression severity. Our results highlight the importance of network synchronization between attention, cognitive control, reward systems in predicting future recovery in late-life depression.

**Keywords:** Functional MRI (fMRI), Geriatric, Antidepressant
**Disclosure:** Nothing to disclose.

### T70. Kainate Receptor Auxiliary Subunit NETO2 Regulates Fear Expression and Extinction

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**Background:** NETO1 and NETO2 are auxiliary subunits of kainate receptors (KARs). They interact with native KAR subunits to modulate several aspects of receptor function. Variants in KAR genes have been associated with psychiatric disorders in humans, and some KAR knockout (KO) mice have increased or decreased anxiety-like behavior. We hypothesized that NETO proteins may regulate anxiety through modulation of KARs.

**Methods:** We carried out comprehensive behavioral analysis of adult male and female Neto1 and Neto2 KO and wild-type (WT) mice. We tested anxiety-like behavior in the elevated plus maze (EPM), elevated zero maze (EZM), light/dark box (LD) and open field (OF) tests. We also assessed home cage locomotor activity in the InfraMot system. To measure fear-related behaviors, we carried out contextual and cued fear conditioning and extinction. To identify the cell types that express Neto2, we carried out in situ hybridization in fear-related brain regions. Finally, we measured the abundance of KAR subunits GLUK2/3 and GLUK5 in the synaptosomal fraction of fear-associated brain regions using Western blot.

**Results:** We did not observe differences between the genotypes in the EPM, EZM, LD, or OF tests. In cued fear conditioning Neto2, but not Neto1, KO mice showed higher fear expression and delayed extinction compared to the WT mice. We established that Neto2 is expressed in both excitatory and inhibitory neurons in the medial prefrontal cortex, amygdala, and ventral hippocampus. The relative amount of GLUK2/3 subunit was reduced 20.8% in the ventral hippocampus, 36.5% in medial prefrontal cortex, and 29.3% in amygdala in Neto2 KO compared to the WT mice. The corresponding reductions of the GLUK5 subunit were 23.8%, 39.5%, and 16.9%.

**Conclusions:** Our results demonstrate that Neto2 regulates fear expression and extinction. Neto2 KO mice showed higher freezing levels already during fear conditioning, compared to WT controls, suggesting increased conditionability, a phenotype that may be related to PTSD-like behavior. This behavioral phenotype may be mediated by KAR subunit abundance at synapses of fear-associated brain regions.

**Keywords:** Behavior, Mouse Model, Kainate Receptor, Fear Conditioning
**Disclosure:** Nothing to disclose.

### T71. Cognitive Flexibility Predicts Emerging PTSD Symptoms and Improves Early Neurocognitive Intervention's Efficacy

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**Background:** Post-Traumatic Stress Disorder (PTSD) is a prevalent, severe and persistent psychopathological consequence of traumatic events. Neurobehavioral mechanisms underlying the early development of PTSD are actively examined. Longitudinally documenting the early neurobehavioral dimensions of trauma exposure may help identify survivors at risk and inform mechanism-based interventions. We present findings from two longitudinal studies that repeatedly probed candidate neurocognitive domains, evaluated their association with emerging PTSD symptoms and their therapeutic modification by early neurocognitive modulation intervention.

**Methods:** Participants in both studies were adult survivors of traumatic events admitted to general hospitals' emergency departments (EDs) for treatment of traumatic injuries and enrolled within one month of the traumatic event. The studies used identical clinical and neurocognitive assessments, which included an assessment of PTSD severity using the Clinician-Administered PTSD Scale (CAPS) and an evaluation of participants' neurocognitive functioning using a WebNeuro battery involving 11 neurocognitive probes. The first study evaluated 181 trauma-exposed individuals one, six, and 14 months following trauma exposure. The second study randomly assigned 87 recent trauma survivors into a web-based neurocognitive modulation intervention ($n = 50$) or control tasks ($n = 37$) and evaluated all subjects ($n = 87$) one, three and six months after trauma exposure. The main outcome measures were the association between neurocognitive probes' Z-scores (predictor variables) and CAPS total severity scores at different time intervals from the traumatic event (main outcome measure), corrected for multiple comparisons.

**Results:** In the first study, better cognitive flexibility at one-month was associated with lower levels of PTSD symptoms at 14 months ($p = 0.002$). In the second study, the neurocognitive remediation was followed by a larger improvement in cognitive flexibility ($p = 0.019$), and lower six months' PTSD symptoms levels ($p = 0.017$). Intervention-induced improvement in cognitive flexibility positively correlated with clinical improvement ($p = 0.002$).

**Conclusions:** Cognitive flexibility shortly after trauma exposure is a significant predictor of PTSD symptom severity, of better responses to neurocognitive remediation and of a better treatment outcome. Cognitive flexibility is a malleable neurocognitive moderator of PTSD pathogenesis, responsive to mechanism-driven preventive intervention.

**Keywords:** Post Traumatic Stress Disorder, Early Intervention, Cognitive Functioning, Computerized Cognitive Training, Clinical Trial
**Disclosure:** Nothing to disclose.

### T72. Anxiety and Stress Symptoms Predict Hippocampal-Dependent Contextualizing Processes

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**Background:** Growing evidence suggests that contextual processing, or the ability to disambiguate potential threat based on environmental information, may underlie posttraumatic stress disorder (PTSD) and other related pathologies. Hippocampal-dependent processes associated with memory encoding and recall, including pattern separation (PS) and pattern completion (PC), are important components of contextualization capacities. Deficits in these processes could contribute to the impaired safety learning and fear modulation observed in anxiety and stress disorders. In this report, we examine relationships between hippocampal function underlying PS and PC of complex contextual scenes and anxiety/stress symptoms.

**Methods:** Twenty-six healthy adults, ranging in age from 19-41 (M = 26, SD = 6.4) completed a memory task during fMRI scanning to assess hippocampal-dependent PS and PC of complex scenes. Clinical measures, including the State Trait Anxiety Inventory (STAI) and the Clinician Administered PTSD Scale (CAPS), were used to assess anxiety and post traumatic stress.

**Results:** Hippocampal activation was positively associated with PC scores (p = .037, FWE SVC). Furthermore, a regression model with trait anxiety and post traumatic stress symptoms predicting PC scores was significant, [F(2, 19) = 3.69, p = .044], with anxiety and stress symptoms predicting 20.4% of the variance in PC performance. Trait anxiety was a significant contributor to this model (B = -.542, p = .017), with higher levels of trait anxiety predicting poorer PC performance. Post-traumatic stress symptoms did not significantly contribute to the model (B = .312, p = .147).

**Conclusions:** These findings suggest that in healthy adults, higher levels of anxiety and stress symptoms are linked to diminished capacity for pattern completion, which is a hippocampal-dependent cognitive process associated with contextualization and memory. This is consistent with the hypothesis that deficiencies in hippocampal-dependent contextual processing could contribute to anxiety and stress vulnerability. Ongoing data collection will further investigate relationships between anxiety and stress symptoms and hippocampal-dependent contextualization and memory in adults with PTSD.

**Keywords:** Anxiety, Functional MRI (fMRI), Memory

**Disclosure:** Nothing to disclose.

## T73. Neural Markers of Successful In-Scanner Worry Reappraisal in Older Adults

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**Background:** Severe worry is a transdiagnostic symptom that is particularly pernicious for the health of older adults. Encountered often in the absence of a Generalized Anxiety Disorder diagnosis, severe worry is associated with increased risk of conversion to Alzheimer's disease and increased risk of cardiovascular events, including stroke. While treatments like cognitive-behavioral therapy are effective at reducing overall anxiety, they are largely ineffective in reducing worry severity in older adults. In this study we investigate the neural basis of both induction and reappraisal of worry using an in-scanner personalized worry task.

**Methods:** We recruited 36 individuals age 50 and older, with varying degrees of worry severity (as measured by the Penn State Worry Questionnaire, PSWQ). Based on the clinical interview with each participant, we built a participant-specific list of worry induction and worry reappraisal sentences. These sentences (one per block) were presented to participants during functional magnetic resonance imaging (fMRI) with fixation in between blocks. Worry induction blocks were followed either by neutral blocks (control condition, included random factual sentences - e.g., "fish live in the ocean") or by worry reappraisal blocks. Following each block, participants rated their worry severity (1-5). After motion correction, spatial normalization to a standard anatomical space, and smoothing we conducted mass-univariate general linear modeling to model worry induction, reappraisal, and neutral blocks. We then parametrically modulated each block by the in-scanner worry severity rating in order to identify regions of activation associated with worry ratings following induction/reappraisal. We also modeled 6 parameters of motion and the mean, along with an autoregressive, AR(1), model to account for unmodelled/aliased noise and a discrete high-pass filter (1/128 Hz) to account for drift. Using statistical non-parametric mapping (SnPM12), we performed a paired t-test to identify regions that were significantly more parametrically modulated during worry induction compared to worry reappraisal. We also conducted paired t-tests to identify regions that were activated more during worry induction and reappraisal compared to neutral. SnPM computes non-parametric p-values which are then corrected using a cluster-wise inference method (cluster forming threshold of $p < 0.001$) that controls the family wise error rate (FWE) at $\alpha = 0.05$.

**Results:** Compared with the neutral condition, worry induction and reappraisal were associated with significantly higher activation in multiple regions including the visual cortex, caudate, the cingulate and prefrontal cortex, hippocampus/parahippocampus, insula, and supramarginal gyrus. The parametric modulation analysis showed that the dorsal anterior cingulate (dACC: x = 6, y = -4, z = 42, Tpeak = 4.7, 171 voxels) and the bilateral temporoparietal junction (supramarginal gyrus SMG: x = -48/56, y = -38/-30, z = 22/16, Tpeak = 4.5, 258/165 voxels) had greater activation associated with higher in-scanner worry severity rating following worry induction. The same regions had greater activation associated with lower in-scanner worry severity rating following worry reappraisal.

**Conclusions:** The dACC and the temporoparietal junction showed greater activation during worry induction when participants reported higher levels of worry at the end of worry-induction blocks. This result may indicate an increased level of affective mentalizing (SMG) coupled with a more sustained effort toward implicit-controlled regulation (dACC) in participants who exhibit higher level of worry following induction.

In contrast, the activation in these regions was greater during reappraisal if the participants reported lower levels of worry at the end of worry-reappraisal blocks. This result may be interpreted as a marker of successful reappraisal and points toward to role of both dACC and SMG in cognitively regulating worry severity. These results indicate potential targets for future interventions designed to alleviate severe worry in older adults (e.g. multimodal interventions such as CBT plus device-based interventions).

**Keywords:** Anxiety, Reappraisal, Worry, Imaging

**Disclosure:** Nothing to disclose.

## T74. Effect of Exogenous Administration of Estradiol on Resting-State Functional Connectivity in Women

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**Background:** Sex hormones contribute to brain sexual dimorphism and functional differences between men and women. Several studies now show that endogenous fluctuations and exogenous administration of estradiol (E2) modulate conditioned fear extinction memory in women. For example, women in the early-follicular phase of the menstrual cycle (low estrogen) show impairment in extinction retention compared to women in the late-follicular phase (high estrogen). We have previously shown that there are sex differences in amygdala subnuclei (centromedial CM and basolateral BL) resting-state functional connectivity (RSFC) and that the difference may be mediated by estrogen availability. Here, we examined the effects of estradiol administration on RSFC in women using a seed-to-voxel approach.

**Methods:** Resting-state (RS) scans from 24 women on oral contraceptives recruited from an ongoing 3-day study that

included fear conditioning (day 1) fear extinction learning (day 2) and extinction recall (day 3) were used in the current analysis. 2 RS scans were obtained from each subject: the first (RS-1) after fear-extinction training and the second (RS-2) a day later (after extinction-recall). Women received an estradiol pill or placebo approximately 5 h before the first RS scan to specifically induce increase in E2 levels during the time window of the scan. No pills were administered during the second RS scan to allow return of E2 to baseline levels before the scan. Blood samples were taken on day 2 and 3 to measure E2 levels. In this current work, our analyses were focused on a seed-to-voxel approach. For the choice of seeds, we selected seeds that are part of the fear network: the ventromedial prefrontal cortex (vmPFC), subgenual anterior cingulate cortex (sgACC), dorsal anterior cingulate cortex (dACC), bilateral posterior hippocampus, bilateral anterior hippocampus, the BL amygdala nuclei, and the CM amygdala nuclei. In the first part of the analysis we divided subjects into two groups: high E2 and low E2 groups based on a median split of their peak E2 levels on day 2. In the second part, we conducted a whole-brain regression analysis using peak E2 level as a covariate of interest. RS scans were conducted in a 3 Tesla GE Discovery System with a 32-channel head coil. Connectivity analyses were performed using the Functional Connectivity (CONN) toolbox that employs procedures from SPM12 software. Significance levels for all data reported in the preliminary results below are reported at uncorrected $p < 0.001$.

**Results:** In the high E2 group, we observed significantly increased coupling between the vmPFC and the dACC on RS-1 compared to RS-2. The dACC showed significant increase in RS coupling with the insular cortex on RS-2 compared to RS-1. Significantly increased functional coupling was also observed between the posterior hippocampus seed and the sgACC on RS-2 compared to RS-1. When comparing groups on extinction recall test we found that the low E2 group had higher connectivity between two dACC seeds and bilateral hippocampus and vmPFC compared to the high E2 group. Regression analysis with E2 levels on RS-1 showed that lower E2 levels are associated with enhanced coupling between the dACC seed and the vmPFC.

**Conclusions:** When estradiol levels are high, there are only few significant changes in resting-state connectivity between areas of the fear network, specifically between the dACC and vmPFC. When estradiol levels are low, however, changes in dorsal anterior cortex connectivity become the most predominant. The changes in the coupling between dACC, insular cortex and hippocampus, areas critical for fear expression, suggest a strength in coupling that may be biased towards fear expression when E2 is low. Our preliminary results suggest that higher levels of E2 may have a "protective role" during resting state in women whereas lower levels of E2 may confer a risk to more expression of anxiety and fear. Future studies and analyses will test this possibility.

**Keywords:** Resting State Functional Connectivity, Estradiol, Women's Mental Health

**Disclosure:** Nothing to disclose.

### T75. Threat Perception, Avoidance and Explicit Memory Following Fear Learning: Distinct Generalization and Neural Activation

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**Background:** Fear generalizes to stimuli that are perceptually similar to learned threats (Lissek et al., 2008). Most generalization research related to anxiety disorders focuses on fear generalization; however, it is unclear whether multiple threat-related appraisals (e.g., threat perception, avoidance, and explicit threat memory) generalizes similarly or distinctly. Based on prior work (Britton, et al., 2017), we hypothesized different generalization patterns would emerge for different threat-related appraisals. In other words, the degrees to which threat perception, threat avoidance and threat memory generalize to perceptually similar stimuli following fear learning would show different patterns. Different generalization gradients would be evident in behavioral appraisals (Study 1) and in neural activation (e.g., hippocampus, ventromedial prefrontal cortex, vmPFC, Study 2).

**Methods:** Healthy adults (age 18-30 years old) completed a fear learning paradigm followed by a generalization paradigm behaviorally ($n = 39$, 23 females) or in the MRI scanner ($n = 25$, 11 females). In the fear learning paradigm (Britton et al., 2017), one neutral face (CS+) was paired with an aversive scream (US), whereas another neutral face (CS-) was never paired with the US. Following the fear learning paradigm, participants rated generalized stimuli, which merged the CS- and CS+ in 10% increments from 0% threat (CS-) to 100% threat (CS+). Participants provided subjective threat perception (current fear), threat avoidance (future probability of avoidance), and ratings of threat memory (past likelihood of scream) in response to generalized stimuli. We compared the linear increase in subjective responses to generalized stimuli across rating types. In a separate sample, we also compared generalization of neural responses supporting these threat-related processes using whole-brain analysis with a combined voxel-wise ($p < 0.001$) and 27-voxel cluster level correction (FWE $p < 0.05$). Finally, brain-behavior relationships were identified using correlations between activation within neural regions identified by whole-brain analyses and area under the curve (AUC) metrics of subjective generalization.

**Results:** Across both studies, generalization of threat perception, threat avoidance and threat memory diverged following fear learning. As expected, all subjective ratings followed a curvilinear pattern, with increasing gradient from the CS- to the CS+. However, the generalization gradient was weakest for threat perception, intermediate for threat avoidance, and strongest for explicit threat memory (all linear comparisons: $F > 3.39$, $p < 0.07$). Deactivation of several parieto-occipital regions (i.e., cuneus, angular gyri, lingual gyrus) followed a generalization gradient for the explicit threat memory condition only; generalization of parieto-occipital activation was absent when appraising threat perception or threat avoidance (FWE $p < 0.05$). Averaged across generalization stimuli, threat-related processes elicited differential insula activation, vmPFC activation, and hippocampal deactivation, that mimicked the behavioral generalization patterns in terms of strength (i.e., threat perception < threat avoidance < threat memory) (FWE $p < 0.05$). Moreover, average neural activation within these regions exhibited unique associations with subjective generalization of threat perception (bilateral insula; both r's > 0.48, both p's < 0.02), threat avoidance (vmPFC; $r = 0.47$, $p = 0.02$), and threat memory (right hippocampus; $r = -0.41$, $p < 0.05$).

**Conclusions:** Patterns of generalization and neural activation patterns differed based on the subjective appraisal, suggesting these processes are unique. Like past research (Britton et al., 2017), individuals differentiated the memory of the CS- and CS+ to a greater extent than perceiving threat. Here, we have also differentiated avoidance and fear in terms of subjective appraisals, demonstrating a greater CS+ and CS- distinction when appraising threat avoidance than threat perception. In addition, appraising threat perception, threat avoidance and threat memory differentially recruited regions commonly identified in fear generalization (i.e., anterior insula, vmPFC, and hippocampus). These diverging patterns could be explained by a combination of factors (e.g., explicit knowledge vs. episodic information, past memory vs. future memory). Moreover, these regions were associated preferentially with a different type of appraisal consistent with

their function. For example, greater generalization gradient of threat appraisal was associated with greater activation of the bilateral insula, a region involved in salience detection. The brain-behavior relationship between vmPFC and generalization of threat avoidance may be explained by emotion regulation; whereas, the link between the hippocampus and the generalization of threat memory may be its involvement in pattern discrimination and explicit knowledge retrieval. Additional work is needed to understand how these threat-related processes work together after fear learning to inform behavior in both adults and children. In particular, dysregulated fear generalization plays a key role in anxiety disorders (Lissek et al., 2010, 2014); therefore, differences in generalization among threat-related processes may ultimately offer important clinical implications.

**Keywords:** Fear Conditioning, Avoidance, Cognitive Appraisal

**Disclosure:** Nothing to disclose.

### T76. Functional Connectivity of Voice Hearing in Women With Posttraumatic Stress Disorder

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**Background:** Voice hearing (VH), or hearing voices in the absence of external stimuli, is most commonly associated with schizophrenia. However, it is a transdiagnostic experience that can also occur in non-psychotic disorders such as posttraumatic stress disorder (PTSD) and dissociative identity disorder (DID). We sought to investigate functional connectivity (FC) abnormalities associated with a lifetime history of VH in treatment-seeking women with PTSD.

**Methods:** 73 women with PTSD were recruited from inpatient, residential, and partial programs specializing in the treatment of women with trauma and related disorders at McLean Hospital. To be eligible, participants had to be female and 18-89 years in age, have legal and mental competency, meet criteria for PTSD, and have a history of childhood abuse. Exclusion criteria included a diagnosis of schizophrenia or other psychotic disorders, alcohol or substance dependence or abuse in the last month, medical or neurological conditions that may cause significant psychiatric symptoms, and MRI contraindications.

We administered the Clinician Administered PTSD Scale for DSM-5 (CAPS-5) to diagnose PTSD, the Structured Clinical Interview for DSM-IV Dissociative Disorders Revised (SCID-D-R) to diagnose DID, and the SCID for DSM-IV-TR to assess for any other Axis I disorders. Participants also completed the Multidimensional Inventory of Dissociation (MID) (Dell, 2006), a comprehensive self-report instrument consisting of 218 items, including 23 different dissociative symptoms. We investigated the rate of VH by determining the number of patients meeting threshold for the auditory hallucination item (B16) in the SCID. Three patients did not complete the SCID, and subsequent analyses were performed in the 70 patients for whom SCID data were available.

We acquired high-resolution structural scans and resting state blood oxygenation level dependent (BOLD) images (124 volumes, TR/TE 3000 ms/30 ms) on a 3 T Siemens Tim Trio scanner at McLean. We used CONN v18a for FC analysis. In addition to standard preprocessing, we used the ARtifact detection Tool (ART, www.nitrc.org/projects/artifact_detect) to identify outlier time points, and included only individuals with < 20 motion outliers. We performed rigorous denoising, regressing out 6 motion parameters and their first derivatives, 5 principal components of white matter signal, 5 principal components of CSF signal, and outlier time points. Images were band pass filtered (0.008, 0.09 Hz). For group-level analysis, we compared VH patients to non-voice hearing (NVH) patients, adjusting for motion outliers and total medication load (TML) (Phillips et al., 2008). In total, 28 VH and 24 NVH PTSD patients were included in FC analysis (BOLD scans available for 28 of 33 VH and 26 of 37 NVH patients; 2 additional NVH patients excluded for > 20 motion outliers). We performed ROI-to-ROI analysis, looking at BOLD time-course correlations across 48 cortical and 21 subcortical regions of the Harvard-Oxford atlas and 26 cerebellar regions from the Automated Anatomical Labeling (AAL) atlas, using a significance threshold of $p < 0.05$, FDR-corrected at the seed level.

**Results:** We found VH to be prevalent in women with PTSD at McLean. Thirty-three (47.1%) of 70 PTSD patients reported a history of auditory hallucinations in the form of VH. All 52 patients met DSM-5 criteria for PTSD; 18 (64%) of the VH patients and 11 (45%) of the NVH patients also met criteria for DID. When comparing resting state FC between the VH and NVH groups, we found VH PTSD patients to have reduced FC within multiple brain region pairs, including left supplementary motor area (SMA) and bilateral planum polare (PP) (left PP $t = -4.0$, p-FDR = 0.029; right PP $t = -3.8$, p-FDR = 0.029); left amygdala (AMY) and right caudate (CAU) ($t = -3.9$, p-FDR = 0.048); left orbitofrontal cortex (OFC) and right parietal regions (supramarginal gyrus $t = -4.0$, p-FDR = 0.038; superior parietal lobule $t = -3.5$, p-FDR = 0.040); and left OFC and right fusiform cortex (FUS) (anterior FUS $t = -3.5$, p-FDR = 0.040; posterior FUS $t = -3.6$, p-FDR = 0.040). VH PTSD patients also showed increased FC between left Heschl's gyrus and regions of the cerebellar vermis (VER) (VER 7 $t = 4.4$, p-FDR = 0.011; VER 6 $t = 4.1$, p-FDR = 0.013); left PP and right occipital cortex (OCC) ($t = 3.6$, p-FDR = 0.042); and between right occipital fusiform gyrus and bilateral insula (INS) (right INS $t = 4.5$, p-FDR = 0.008; left INS $t = 3.7$, p-FDR = 0.046). When we adjusted for dissociative symptoms, as measured by mean MID score, the findings of reduced left AMY-right CAU FC and increased left PP-right OCC FC were no longer statistically significant, while we found additional FC abnormalities, specifically increased right cuneus-right inferior frontal gyrus FC ($t = 3.9$, p-FDR = 0.048) and increased left OFC-left putamen FC ($t = 3.6$, p-FDR = 0.034) in VH patients, relative to NVH patients.

**Conclusions:** Abnormal FC involving brain regions involved in auditory, speech, and language processing (e.g., Heschl's gyrus, PP, supramarginal gyrus) are consistent with inner speech models of VH. Reduced FC between frontal control areas (e.g., OFC, SMA) and temporal and parietal regions also suggest deficient top-down modulation of speech/language areas. Abnormal FC involving limbic regions (e.g., AMY) may also point to a memory based model of VH, which may be particularly relevant in PTSD. A growing literature suggests that VH may be mediated by dissociation. However, the majority of our findings remained even after adjusting for dissociative symptoms.

**Keywords:** Auditory Hallucinations, Posttraumatic Stress Disorder, Resting State Functional Connectivity

**Disclosure:** Nothing to disclose.

### T77. Altered Connectivity Between Default Mode and Fronto-Parietal Networks in Unmedicated Adults With OCD

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**Background:** Resting state functional MRI differences between adults with and without OCD are beginning to be characterized. However, much existing literature has focused on specific regions of interest (ROIs) defined a priori from structural or task-based

functional studies, leaving little room to detect abnormalities outside traditionally-studied networks. Furthermore, due to logistical difficulty in recruiting unmedicated patients, many imaging studies suffer from a confounding effect of psychotropic medications on brain function. Here, we collected resting state scans in a sample of medication-free adults with OCD. We analyzed whole-brain differences in connectivity between patients and controls using a network-based statistic (NBS) method and assessed how these brain networks correlated with OCD symptom severity.

**Methods:** Thirty adults with OCD and 31 age and sex matched healthy controls were recruited to complete T1-weighted and 2 5-minute resting state functional MRI scans (TR 2200 msec, TE = 30 msec, 3.75 × 3.75 × 3.5 mm effective resolution) on a GE SIGNA 3 T scanner. All participants were assessed for OCD symptoms on the day of the MRI scan using the Yale–Brown Obsessive Compulsive Scale (Y-BOCS; Goodman, et al., 1989). Fifty-one participants additionally completed a computerized neuropsychological task, the Conner's Continuous Performance Test II (CPT-II; Conners and Staff, 2000), which is widely used to measure attention. All participants, including those with OCD, were psychotropic medication-free for at least 8 weeks prior to the MRI scan.

Resting state images were preprocessed in SPM and the Conn toolbox following standard procedures, including regressing out frames with frame-to-frame head motion > 0.2 mm or frame-to-frame changes in global-signal z > 2. Participants with less than 5 minutes of resting state data remaining after outlier regression were removed from analyses, leaving 24 patients (12 male, age = 29 ± 8 years; 22 with CPT-II data) and 24 controls (12 male, age = 28 ± 8 years; 22 with CPT-II data).

The brain was segmented into 160 ROIs based on a previously-published brain atlas (Dosenbach, et al., 2010). Correlations were calculated between each pair of ROIs and Fisher r-to-z transformed. The NBS (Zalesky, et al., 2010) method was implemented in NBS Connectome, which uses permutation testing to control for multiple comparisons. Analyses examined group differences and relationships with symptom severity, including mean framewise displacement as a covariate to control for residual effects of participant head motion. NBS results were qualitatively similar with and without age and sex as covariates, as well as with extent and intensity thresholding.

Group differences in CPT-II performance were examined using unpaired two-sample t-tests with unequal variance in Matlab. Mediation analyses were performed using the 'mediation' package in R.

**Results:** NBS analyses revealed one network exhibiting significantly greater connectivity in patients relative to controls ($p = 0.009$). This network consisted of six edges/connections between ROIs/nodes in the left dorsal frontal cortex, left posterior cingulate, left intraparietal sulcus, left thalamus, and bilateral precuneus. NBS also identified one network with significantly greater connectivity in controls relative to patients ($p < 0.001$); this consisted of eight edges primarily between nodes in the occipital cortex.

CPT-II performance differed significantly between patients and controls, with patients showing faster response times on correct trials ("Hit RT"; patient mean T-scaled score = 42.1, control mean = 54.3, $t = -2.58$, $p = 0.014$). Three left hemisphere connections in the first NBS network above that exhibited greater connectivity in patients relative to controls also showed significant negative correlations with Hit RT in the CPT-II (FDR corrected). An exploratory mediation analysis showed that the average of these three functional connectivities significantly mediated group differences in Hit RT (proportion of effect mediated = 59%, $p = 0.02$).

**Conclusions:** Our whole-brain NBS revealed increased connectivity in unmedicated OCD patients between left hemisphere default mode and task-control regions, corroborating previous work focusing on a priori ROIs (Stern, et al., 2012). Patients performed faster than controls on the CPT, potentially indicating greater impulsivity; this pattern of performance was mediated by connectivity across default mode and fronto-parietal networks. These findings suggest that altered connectivity between these networks might contribute to an impulsive response style in OCD. We are currently conducting NBS analyses on a larger sample of OCD patients and controls who were scanned using a multiband imaging protocol before completing a standard course of exposure and response treatment for OCD. Such data will allow us to determine if these left lateralized findings can be replicated and if altered connectivity across default mode and fronto-parietal circuits might be a marker of treatment response in unmedicated OCD patients.

**Keywords:** Obsessive-Compulsive Disorder (OCD), Resting State fMRI, Network Based Statistic (NBS)

**Disclosure:** Nothing to disclose.

## T78. Negative Affects in Anxious Patients With Alcohol Use Disorder

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**Background:** There are a number of stress-based theories of development and maintenance of drug use including alcohol use disorder (AUD) (Brady & Sonne, 1999; Heinz et al 2001; Valdez and Koob, 2004). Studies have shown association between substance use disorders and mood and anxiety disorders (Grant et al, 2004). Compared to neutral faces, amygdala preferentially responds to fearful faces in contrast to neutral faces and habituates rapidly. Anxiety prone individuals have shown greater bilateral amygdala and insula activity to negative emotional faces (Stein et al., 2007). The amygdala response to emotional faces in patients with AUD has been somewhat contradictory. Some studies have shown that anxious patients with AUD show a blunted response to emotional stimuli during fMRI in anterior cingulate cortex, amygdala, and hippocampus (Salloum 2007; Marinkovic, 2009). While, others have characterized patients with AUD as having a greater tendency to experience negative emotion and impulsivity and a lack of social constraint (Elkins et al., 2006; Sher et al., 1999). In this study we have examined the association between an alcohol use severity, anxiety score, and alterations in brain activation during exposure to negative facial emotion stimuli.

**Methods:** Participants in this study were all male and included 21 healthy controls, 18 patients with AUD, and 22 patients with diagnosis for both AUD and clinically significant anxiety symptoms.

We analyzed the association between alcohol use disorder Identification Test (AUDIT) and State-Trait Anxiety Inventory (STAI-T) and investigated the relevant neural substrates of AUD and anxiety using a block design fMRI task.

All participants were administered a Face Matching task modified from Hariri et al., 2002. In this task a target picture is presented at the top of screen and the participant is asked to determine the matching picture from the two pictures shown at the bottom of the screen. Each picture belongs to one of the six categories: angry faces, sad faces, fearful faces, happy faces, neutral faces, and geometric shapes. The task consisted of blocks of six pictures from each category. Each block started with a 2-second long instruction: "match the shapes" or "match the faces". Two blocks for each emotion were presented in a random order, interleaved with a block of abstract shapes. Each face or shape

trial lasts for five seconds during which the participant makes her/his selection by pressing the left or right button. There was no inter-stimulus interval and there was an equal number of male and female faces.

Using a Siemens 3-Tesla Skyra scanner (Siemens Medical Solutions USA, Inc., Malvern, PA) fMRI datasets were collected using a single-shot gradient echo planar imaging pulse sequence with thirty-six axial slices acquired parallel to the anterior/posterior commissural line (TR = 2000 ms, TE = 30 ms, flip angle = 90 degrees, 3.75 mm × 3.75 mm × 3.8 mm voxels).

To investigate the differences in the effect of emotional faces among our three groups we utilized AFNI (Cox, 1996). In this preliminary analysis, we conducted statistical analysis at voxelwise $p = 0.001$ at the cluster level alpha = 0.05.

**Results:** In this study we found that the Pearson's correlation between STAI-T and AUDIT scores were 0.21, 0.07, and 0.38 for healthy controls (CON), AUD without anxiety (NAA), and AUD with anxiety (AA), respectively. Only the correlation between AUDIT and STAI-T of AA was at trend level ($p = 0.08$).

Anxious AUD participants showed significantly higher activation to angry faces in right caudate relative to healthy controls (with decreased activation with respect to baseline).

Anxious AUD showed lower activation (no response) to fearful faces in left cingulate gyrus in comparison to non-anxious AUD.

Non-Anxious AUD showed a trend higher activation in left precuneus in response to fearful faces in comparison to controls.

**Conclusions:** Our preliminary results indicate a stronger association between STAI-T anxiety score and alcohol use severity as measured by AUDIT score in participants with both AUD and anxiety symptoms in comparison to control subjects and AUD participants without anxiety.

The neuroimaging data suggests patients with AUD and anxiety symptoms show hypersensitivity to negative affects as demonstrated by response to angry facial expression stimuli. The hyperactivation of caudate might point to increased valuation of this type of emotions. On the other hand, the hypoactivation of anterior cingulate gyrus may suggest an inability of anxious AUD participants to normalize their response to fearful stimuli. The hyperactivity of AUD in left precuneus demonstrates the potential attention bias across AUD to negative affects.

Further investigation is needed to include female participants as well as control subjects with anxiety diagnosis only to be able to generalize these preliminary findings. Our preliminary data also motivates future studies to investigate the relationship between anxiety and alcohol use and its effects on neuroimaging data.

**Keywords:** Alcohol Use Disorder, Anxiety, Neuroimaging

**Disclosure:** Nothing to disclose.

### T79. Beneficial Effects of FAAH Inhibition on Fear- and Stress-Related Behaviors in Healthy Humans

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**Background:** Dysregulation of stress- and threat-responding can lead to psychopathologies such as post-traumatic stress disorder (PTSD) and related anxiety disorders. The endocannabinoid (eCB) system is proposed to act as a "stress buffer" and thus represents a novel therapeutic target to treat stress-related psychiatric disorders. Exogenous compounds directly targeting cannabinoid (CB) receptors have been associated with unwanted side effects, in part due to indiscriminant activation of CB receptors. However, recent evidence suggests that a more selective approach may instead be to target eCB degradation enzymes to amplify the endogenous, physiologically-relevant eCB signal.

In animal models, inhibition of fatty acid amide hydrolase (FAAH), the main degradative enzyme of the eCB anandamide (AEA), enhances AEA levels, facilitates extinction of conditioned fear, and prevents the anxiogenic effects of stress and other aversive environmental experiences. FAAH inhibitors are not yet commercially approved for human use, but insights into the beneficial effects of elevated AEA in humans have come from the FAAH C385A variant, which encodes a less active FAAH enzyme. Using this approach, we have recently shown that reduced FAAH activity conferred via the FAAH C385A variant A-allele is associated with enhanced baseline AEA and facilitation of fear extinction. Moreover, A-allele homozygotes were protected against stress-induced decreases in AEA and concomitant increases in negative affect. Together, our previous data suggest that enhanced AEA may be a suitable therapeutic target for stress-related psychopathologies. Here, we aimed to evaluate whether pharmacological inhibition of FAAH can produce similar beneficial effects in healthy humans.

**Methods:** Healthy adults (N = 60; 26 M/34 F) were randomized to receive Pfizer compound PF-04457845 (FAAHi; n = 30; 15 M/15 F) or placebo (PBO; n = 30; 11 M/19 F) for 10 days. On days 9 and 10 of dosing, participants complete a 2-day behavioral and psychophysiological laboratory paradigm. In a 2-day fear conditioning paradigm, we assessed fear-potentiated startle via facial electromyography (EMG) of the orbicularis oculi ("eye blink") muscle. Day 1 consisted of fear conditioning and [within-session] extinction, while recall of extinction was assessed on day 2. Affective responses to emotional images (International Affective Picture System; IAPS) were assessed via facial EMG of the corrugator superciili ("frown") and zygomaticus major ("smile") muscles. Subjective, psychophysiological, and biochemical measures were obtained in responses to a standardized stressor (the Maastricht Acute Stress Task; MAST) or control task, completed on separate days. Subsequently, stress-induced changes in affective responses to emotional images were again measured using facial EMG.

**Results:** FAAH inhibition was associated with enhanced fear extinction. All inhibitor-treated participants demonstrated greater recall of extinction on day 2 (effect of treatment: $p = 0.008$, partial $\eta 2 = 0.135$) but only female inhibitor-treated participants demonstrated facilitated extinction on day 1 (e.g. within-session extinction; treatment x time x sex: $p = 0.017$, partial $\eta 2 = 0.099$). There was no effect of FAAH inhibition on psychophysiological or subjective response to stress. However, following stress, FAAH inhibition was associated with reduced negative affect (e.g. corrugator activity, "frowning") to negative stimuli (effect of treatment: $p = 0.010$; partial $\eta 2 = 0.113$). Again, this effect was more robust in female than male participants (treatment x sex: $p = 0.016$, partial $\eta 2 = 0.099$) Analyses are currently underway to assess stress-induced changes in cortisol and circulating endocannabinoids.

**Conclusions:** We report the first evidence of the ability of pharmacological inhibition of FAAH to influence stress- and fear-related behaviors in humans. Interestingly, these effects appear to be more robust in females than in males. Together, this data suggests that elevated AEA via FAAH inhibition represents a novel pharmacotherapeutic target for the treatment of stress-related disorders such as PTSD. Moreover, our data highlights potential sex differences in the efficacy of eCB augmentation that may prove critical in the treatment of human psychiatric disease with this drug class.

**Keywords:** Endocannabinoids, Post Traumatic Stress Disorder, Fear Conditioning, Negative Affect, Human Laboratory Study

**Disclosure:** Nothing to disclose.

## T80. Targeting Anxiolytic and Antidepressant Properties of Low Dose Psychedelic D-Lysergic Acid Diethylamide (LSD) in a Murine Model of Chronic Stress

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**Background:** D-lysergic acid diethylamide (LSD) is a psychedelic drug that has recently generated interest due to clinical findings reporting its beneficial anxiolytic properties [1]. However, until recently, the effects of LSD in areas of the brain related to anxiety and depression have not been deeply studied in animal models. A study carried out in our laboratory [2] has demonstrated that low doses of LSD (5-20 µg/kg) decreased the activity of serotonin (5-HT) neurons in the Dorsal Raphe Nucleus (DRN), which is a region involved in anxiety [3] and depression [4]. However, LSD, at higher doses (60-120 µg/kg), decreased the firing rate of dopaminergic neurons in the Ventral Tegmental Area (VTA), which is involved in psychosis [5], thus suggesting a psychotic-like effect at higher doses, as previously reported in behavioral experiments [6]. A recent in vitro study showed that LSD (0.409 nM) promotes neuritogenesis in rat cortical cell cultures via mammalian target of rapamycin (mTOR) signaling pathway [7], similarly to the non-psychedelic hallucinogen ketamine [8]. The mTOR pathway contributes to the neurobiology of depression and anxiety [9] and can be upstream stimulated by the phosphorylation at serine 473 of the protein kinase B (Akt-Ser473) [10]. Thus, this study examined the effects of repeated administration of low-dose LSD in a mouse model of chronic stress (CS). Behavioural paradigms of anxiety and depression and in vivo electrophysiological recordings of 5-HT neurons in DRN were measured. Finally, given that hippocampus plays a role in anxiety [11] and depression [12], preliminary western blot (WB) analysis was conducted to demonstrate the ability of LSD to modulate the hippocampal phosphorylation of mTOR and Akt-Ser473 proteins in control (CTL) mice.

**Methods:** The CS paradigm was performed by using a modified protocol as previously described [13]: 8-week old male C57BL/6 J mice were individually placed in restrainers for 2 h per day, over 14 days. CTL mice remained undisturbed in their original cages. From the 7th to 14th day of stress, both CTL and CS mice received intraperitoneal (i.p.) injections of LSD (5, 15 or 30 µg/kg/day, i.p.) or vehicle (veh); on the 15th day after the CS, the groups of mice were tested separately. To evaluate anxiety-like behavior [14] the Open Field Test (OFT), Novelty Suppressed Feeding Test (NSFT) and Light Dark Box test (LDBT) were employed. The Forced Swim Test (FST) and the Sucrose Preference Test (SPT) were used to evaluate depressive-like behavior [15]. In-vivo single unit extracellular recordings of 5-HT DRN neurons were also performed, following our standardized protocol [14]. Hippocampal phosphorylation levels of mTOR and Akt-Ser473 in CTL animals were conducted employing WB analysis [16]. Two-way ANOVA and Bonferroni post-hoc comparison was used using stress and treatment factors for the behavioral and electrophysiological experiments. Student's t-test was used for WB analysis in CTL animals.

**Results:** CS mice ($n = 9$) showed less time spent ($p < 0.05$) and fewer entries ($p < 0.05$) in the center of the OFT, compared to the CTL group ($n = 9$). Treatment with LSD (30 µg/kg/day) normalized both parameters to CTL levels ($p < 0.05$). No effect was detected in the distance travelled for treatment ($F\ (3,\ 68) = 0.2805$, $p = 0.8393$), stress ($F\ (1,\ 68) = 0.1915$, $p = 0.6631$) factors and interaction ($F\ (3,\ 68) = 1.987$, $p = 0.1241$). CS mice ($n = 9$) displayed increased latency to feed in the NSFT ($p < 0.05$) compared to CTL mice ($n = 9$), decreased by LSD treatment (30 µg/kg/day) ($p < 0.05$). Furthermore, CS mice ($n = 8$) spent less time in the illuminated area ($p < 0.05$) in the LDBT, compared to CTL ($n = 8$), effect reverted by LSD (30 µg/kg/day) which increased the time spent in the bright area in CS mice ($n = 8$, $p < 0.001$ vs CS mice which received veh). Concerning the despair-like behavior, CS mice ($n = 9$) displayed increased immobility time in the FST ($p < 0.05$) vs CTL ($n = 8$). Treatment with the LSD (30 µg/kg/day) decreased immobility time in CS mice ($n = 11$, $p < 0.05$). No effect was detected in the SPT for treatment ($F\ (1,\ 34) = 0.2675$, $p = 0.6084$) and stress ($F\ (1,\ 34) = 0.7771$, $p = 0.3842$) factors and interaction ($F\ (1,\ 34) = 1.065$, $p = 0.3095$). LSD at 5 and 15 µg/kg/day did not produce any effect in these behavioral paradigms. In vivo electrophysiological recordings revealed that CS mice ($n = 29$ neurons) showed decreased activity of 5-HT neurons in the DRN, (effect of stress factor: $F\ (1,\ 156) = 14.84$, $p = 0.0002$, without interaction: $F\ (1,\ 156) = 0.2609$, $p = 0.6102$), coupled with increased coefficient of variation (effect of stress factor:$F\ (1,\ 155) = 15.37$, $p = 0.0001$, without interaction: $F\ (1,\ 155) = 1.940$, $p = 0.1657$) compared to the CTL group ($n = 40$ neurons). Importantly, LSD (30 µg/kg/day) increased the firing of 5-HT neurons in both CS ($n = 46$ neurons) (effect of treatment: $F\ (1,\ 156) = 19.90$, $p < 0.0001$) and CTL mice ($n = 45$ neurons), paralleled with decreased coefficient of variation (effect of treatment: $F\ (1,\ 155) = 9.149$, $p = 0.0029$). Finally, preliminary WB analysis in CTL animals ($n = 5$) revealed that LSD (30 µg/kg/day) increased hippocampal phosphorylation of both mTOR ($p < 0.05$) and Akt-Ser473 ($p < 0.05$) proteins.

**Conclusions:** This study provides confirmation that LSD at low doses prevents the development of anxious and depressive behavior after CS, normalizes the 5-HT DRN activity in stressed mice and increases the mTOR and Akt phosphorylated proteins in physiological conditions.

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**Keywords:** LSD, Dorsal Raphe Serotonin Neurons, Anxiety, Electrophysiology, Chronic Stress

**Disclosure:** Nothing to disclose.

### T81. Neural and Behavioral Correlates of Approach-Avoidance Conflict in a Large Transdiagnostic Sample

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**Background:** Anxiety disorders are among the most prevalent mental health disorders and are highly comorbid with depression (Kessler, 2005), resulting in significant individual and socio-economic burden. Given the high rates of symptom renewal in these disorders, effective treatments are highly needed. Approach-avoidance conflict refers to the simultaneous activation of avoidance and approach motivations during decision-making and has significant transdiagnostic and translational relevance to our understanding and treatment of anxiety. Previous research in healthy humans has shown that relative to males, females exhibit less approach behavior during approach-avoidance conflict (Aupperle, 2011), and that attenuated approach behavior may be driven by anxiety sensitivity and/or behavioral activation drives. Previous neuroimaging work suggests that a network involving anterior cingulate cortex (ACC), dorsolateral prefrontal cortex, insula, and caudate regions may be involved in processing and responding to approach-avoidance conflict (Aupperle, 2014). Here, we set out to replicate these findings and extend them to clinical populations. Additionally, we examine the neural correlates of approach-avoidance conflict in a sample of anxious and depressed patients currently undergoing psychological treatment.

**Methods:** As part of Tulsa 1000, an ongoing, naturalistic longitudinal study based on NIMH RDoC framework (Victor et al., 2018), 311 individuals (57 healthy controls and 254 with mood and anxiety disorders) completed the approach-avoidance conflict (AAC) task (Aupperle, 2011). Participants were asked to decide between negative and positive affective image/sound pairs shown on opposite sides of a runway. In conflict trials, negative stimuli were paired with reward points, while positive affective stimuli were not. Participants also rated their motivation to approach rewards and avoid threats and completed the Overall Anxiety Severity and Impairment Scale (OASIS). As part of an ongoing clinical trial, 47 patients with mood and anxiety disorders completed the AAC task while undergoing functional magnetic resonance imaging (fMRI). Linear mixed effects models were used to examine condition by gender effects on AAC task behaviors, while independent t-test and Pearson's coefficients were used for post-hoc analyses. For fMRI analysis, voxel-wise, whole-brain mixed effects multilevel regression analysis identified conflict vs non-conflict (avoid-threat and approach-reward) condition differences, considered significant at pcorr < .005.

**Results:** Across all participants, there was a main effect of condition [$F(4,1236) = 508.29$, $p < .001$] and Gender [$F(1,309) = 11.07$, $p < .001$] on behavior, qualified by a gender by condition interaction [$F(4,1236) = 3.95$, $p < .01$]. While males and females did not differ on approach-reward [$t(309) = -1.70$, $p = .08$] and avoid-threat trials [$t(309) = -1.26$, $p = .21$], males exhibited greater approach behavior on conflict trials than females [$t(94) = -3.06$, $p < .01$; Cohen's $d = .38$]. This was accompanied by greater self-reported motivation by males to approach rewards [$t(309) = -2.38$, $p = .02$; Cohen's $d = .29$]. Relative to healthy females, anxious and depressed females showed greater approach behavior on conflict trials [$t(215) = -2.33$, $p = .02$; Cohen's $d = 1.70$]. Greater OASIS scores related to less approach behavior in healthy females [$r(28) = -.52$, $p < .01$], but not in anxious and depressed females [$r(183) = -.03$, $p = .67$; $z = -2.65$; $p < .01$]. There was no effect of diagnosis on approach behavior in males [$t(94) = -.02$, $p = .98$]. In mood/anxiety patients, conflict trials elicited greater activation compared to non-conflict trials within bilateral ACC and left medial frontal gyrus.

**Conclusions:** The results of this large transdiagnostic study partially replicate prior work examining behavioral and neural correlates of approach-avoidance conflict. Similar to previous findings, males and females generally did not differ on approaching rewards and avoiding threats, but females were less likely to exhibit approach behavior during conflict (i.e., where approaching rewards carried some probability of negative outcomes). Contrary to previous studies in subclinical populations, depressed and anxious females exhibited greater approach behaviors during approach-avoidance conflict than healthy control females. Symptoms of anxiety appeared to influence approach behavior in healthy, but not in females with anxiety and mood disorders. Neuroimaging results further confirm the central role of the ACC in approach-avoidance conflict. Future studies will examine how behavioral and neural responses during conflict situations may contribute to one's propensity for treatment response, as well as how they may change with completion of psychological treatment.

**Keywords:** Approach/Avoidance, Conflict, Anxiety

**Disclosure:** Nothing to disclose.

### T82. Rapamycin Improves Social Deficits in the Mice Treated Prenatally with Valproic Acid

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**Background:** Autism spectrum disorder (ASD) is a developmental disability that is characterized by social deficits. Various genetic and environmental factors have been investigated in studies for ASD. However, the efficient medical treatment for impairment of social interaction in ASD has not been established. Valproic acid (VPA) is used as an anti-epileptic drug and a mood stabilizer. Pregnant women treated with VPA deliver their children with ASD. Exposure to VPA prenatally in animals has been used to ASD model. Recent studies showed that aberrant mTOR signaling pathway causes ASD-like behaviors in VPA-exposed animals. The

mTOR signaling pathway regulates neuronal cell proliferation and synaptogenesis. Over activation of the mTOR signaling has been implicated in the pathogenesis of syndromic ASDs, such as tuberous sclerosis complex (TSC). Treatment with rapamycin, mTOR complex inhibitors, improves social deficits in Tsc heterozygous mice. These studies suggest that over activation of mTOR signaling pathway is involved in ASD and mTOR inhibitor is a potential therapeutic drug. Therefore, our aim is to clarify the effect of rapamycin treatment in social deficits in VPA-exposed animals.

**Methods:** We injected subcutaneously VPA (600 mg/kg) into female mice at gestation 12.5 day and used the pups as ASD model. The pups were injected with rapamycin or vehicle once daily for 2 consecutive days and social interaction test was conducted after administration of rapamycin in ages 5-6 weeks (adolescence) or 10-11 weeks (adult) mice. The mouse whole brains were obtained after the social interaction test on adult, and microarray and western blots analysis were performed. All of the animal experiments were performed in accordance with the Guidelines for the Care of Laboratory Animals of the Tokyo Metropolitan Institute of Medical Science, and the housing conditions were approved by the Institutional Animal Care and Use Committee.

**Results:** We found that mice that were prenatally exposed to VPA and treated with vehicle exhibited impairment of social interaction compared with control mice that were treated with vehicle. Rapamycin treatment in VPA-exposed mice improved social deficits in both adolescence and adult. Mice that were prenatally exposed to VPA and treated with vehicle exhibited the aberrant expression of genes in the mTOR signaling pathway, and rapamycin treatment recovered changes in the expression of some genes, including Fyb and A330094K24Rik. Moreover, rapamycin treatment suppressed S6 phosphorylation in VPA-exposed mice.

**Conclusions:** These results suggest that administration of rapamycin is effective for treatment of non-syndromic ASD that is caused by aberrant mTOR signaling pathway.

**Keywords:** Autism Spectrum Disorder, Rapamycin, Mice, Valproic Acid

**Disclosure:** Nothing to disclose.

### T83. Dysfunctional Reward Valuation in Social Interactions Following Exposure to Early Life Stress

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**Background:** Exposure to early adversity is associated with both maladaptive social relationships and increased drug use. Under normal conditions, social behavior and drug use is rewarding and reaches a peak during adolescence before declining in adulthood in both males and females. However, reward insensitivity is observed in individuals exposed to early life stress. As reward value is partially encoded by the D1 dopamine receptor in the prefrontal cortex, the insensitivity can be explained by our observations of reduced D1 receptors on prefrontal projections to the accumbens in animals exposed to early life stress. However, we also observed increased D1 on prelimbic afferents in early stressed animals. In the current study, we investigated the role of prefrontal D1 and its relationship to social reward processing during development in rats exposed to early life stress.

**Methods:** We used maternal separation (MS) paradigm as a species-relevant early life stressor to investigate sex-dependent changes in social behaviors in juvenile and adolescent male and female rats (n = 7-9 subjects/group). Empathy was tested with conspecifics and novel subjects (n = 6/group), and two aggression tests were used: social interaction and resident intruder. The role of D1 on prelimbic afferents was investigated via overexpression of the D1 receptor with lentivirus in juvenile rats were D1 is normally low (n = 7/subjects/group). Data were analyzed by ANOVAs.

**Results:** MS males exhibited more aggression ($F1, 24 = 6.73$, $P = 0.02$) and MS females more affiliative behavior ($F1, 24 = 5.15$, $P = 0.03$) than sex-matched controls. Juvenile males with elevated D1 receptors also demonstrated more aggression ($F1, 14 = 4.5$, $P < 0.05$) and increased social interactions to familiar, but not novel, conspecifics (Virus X Social Test: $F1, 11 = 13.97$, $P = 0.003$). Aggression was not observed in the resident intruder paradigm.

**Conclusions:** Elevated levels of D1 on glutamate outputs in MS males produce dysfunctional social interactions that are recapitulated by elevating D1 via viral-mediated transfer. The nature of elevated aggressive behavior in MS males was more specific to a familiar, and not a novel rat, and also not due to territorial threat. Ongoing studies are investigating which downstream targets are modulated by D1-expressing prelimbic afferents. These data suggest that early life stress modulates reward valuation via D1 receptors to produce aggression that may have relevance to conduct disorder.

**Keywords:** Aggression, Early Life Stress, Sex Differences, Social Behavior

**Disclosure:** Nothing to disclose.

### T84. Temporal Relationships Between the Change in Cannabis Use and Depressive Symptoms in Youth Following Evidence-Based Psychosocial Interventions for Cannabis Use Disorder

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**Background:** Cannabis use disorder (CUD) and depression frequently co-occur in youth. Clinically significant depressive symptoms and current major depressive disorders are present in 20-30% of adolescents diagnosed with a CUD. The relationships between cannabis use and depression are complex. Depressive symptoms may increase or decrease in cannabis users over time and significant variability exists within individuals across time-periods (e.g. during acute intoxication vs. short term vs. long-term time windows). The presence of co-occurring depression in youth treated for CUD may affect both cannabis use and depression treatment outcomes. How depressive symptoms change over the course of CUD treatment and whether cannabis use and depression change concurrently vs. sequentially is unknown. Improved understanding of the temporal characteristics of cannabis-depression associations during CUD treatment has the potential to improve clinical management for youth with co-occurring disorders by guiding the timing and sequencing of therapeutic content. In the present study, we examine the strength and temporal dynamics of longitudinal associations between cannabis use and depressive symptoms following receipt of evidence-based treatment for CUD in a large adolescent sample using data from the Cannabis Youth Treatment Study (CYT), a multisite clinical trial (Dennis et al., 2004).

**Methods:** Six hundred adolescents (ages 12-18 years) with a DSM-IV CUD were randomly assigned to receive up to 12 weeks of CUD treatment from one of five psychosocial interventions. These interventions included motivational enhancement therapy/cognitive behavioral therapy (MET/CBT) 5-session (MET/CBT-5) and 12-session (MET/CBT-12) versions, Family Support Network (FSN),

Adolescent Community Reinforcement (ACRA), and Multidimensional Family Therapy (MDFT). All participants completed the Global Appraisal of Individual Needs (GAIN) at five time-points: baseline (BL) and at 3-months, 6-months, 9-months, and 12-months post-baseline. Cannabis use was assessed via calendar method (past 90 days) and the GAIN-Depression Symptom Inventory was used to assess depressive symptoms. Of the 600-youth randomized at BL, 597 (99%) had one or more follow-ups and 568 (95%) had all four follow-ups.

A bivariate latent change model assessed bidirectional effects of baseline levels and time-lagged changes in depressive symptoms and cannabis use on depression and cannabis use outcomes.

**Results:** Depressive symptoms (70.2%) and MDD (18.0%) were common in adolescents with a CUD at BL. Both cannabis use and depressive symptoms decreased significantly following CUD treatment. The model fit indices indicate that, overall, the model fit the data well. Between subjects, change in cannabis use was significantly associated with change in depressive symptoms over the study period ($b = 1.22$, $p = 0.003$). The severity of depression and frequency of cannabis use at BL were also associated with change rates, whereby youth with higher depression and more frequent cannabis use at BL had larger reductions in depressive symptoms and cannabis use. Time-lag analyses showed that within-subjects the severity level of depressive symptoms (from one time point to compared to the next) was predicted by previous depressive symptoms ($b = -0.71$, $p < 0.001$) but not cannabis use ($p = 0.068$) and the frequency of cannabis use was predicted by previous cannabis use ($b = -1.47$, $p < 0.001$) but not depressive symptoms ($p = 0.158$) respectively.

**Conclusions:** A clinically meaningful longitudinal relationship between decreasing cannabis use and decreasing depression is present among adolescents enrolled in CUD treatment. These findings suggest that evidence-based outpatient psychosocial interventions for youth with CUD provide benefit for reducing both cannabis use and depressive symptoms and that rates of change of cannabis use and depression during treatment are coupled. Furthermore, our findings on temporal dynamics indicate that cannabis use and depressive symptoms decrease concurrently (as opposed to in a staggered fashion) following CUD treatment. In context of the parallel and concurrent pattern of change in cannabis use and depressive symptoms, providers should use integrated treatment protocols and consider increasing the intensity of treatment for youth whose depressive symptoms or cannabis use do not initially improve with psychosocial treatments. Future studies should consider characterizing treatment-related cannabis-mood relationships by examining real-time changes in cannabis use, depressive symptom clusters, and negative and positive valence domains using a Research Domain Criteria (RDoC) framework.

**Keywords:** Adolescents, Cannabis Use Disorder, Depression, Psychosocial Treatment, Treatment Response

**Disclosure:** Nothing to disclose.

### T85. Single Dose Drug Challenge Study in Fragile X Syndrome: Initial Report of Drug Impact on EEG Markers

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**Background:** Fragile X Syndrome (FXS) is the most common single gene cause of autism and the most common inherited form of intellectual disability. Persons with FXS exhibit abnormalities on EGG including enhanced resting state gamma band activity and reduced habituation to repeated auditory stimuli. We have been conducting a single dose challenge study of acamprosate, lovastatin, minocycline, and placebo in adolescents and adults with FXS in order to evaluate the impact of these targeted treatments on clinical and electrophysiologic markers.

**Methods:** We are enrolling thirty 15 to 50-year olds with full mutation (FXS) in a double-blind, placebo-controlled crossover trial of single dose acamprosate (1,333 mg), lovastatin (40 mg), minocycline (270 mg of extended release formulation), or placebo. There are two-week washout periods between treatment days. Subjects complete full EEG batteries and additional continuous performance computer-based testing (KiTap), and other memory and cognitive batteries before drug dosing and six hours post-dosing.

**Results:** The first 15 of 30 subjects were evaluated to date. Subject mean IQ is 50.8 with a mean age of 25.9 years (range 15-41 years). Minocycline use was associated with enhanced habituation to auditory stimuli. This minocycline-associated rescue of auditory habituation deficits was associated with improvement in auditory attention post-dose as measured by the Woodcock-Johnson. No other drug treatment was associated with change in auditory habituation. No total brain EEG power changes occurred with drug or placebo treatment. A region-specific reduction in gamma power in right temporal cortex was noted with minocycline treatment. Minocycline-associated directional improvements in resting state gamma, N1 habituation, and low gamma band synchronization tracked together. Significant test-retest reliability was noted for all EEG measures from treatment day to treatment day across the study.

**Conclusions:** We are now using these single day pre- and post-dosing quantitative target engagement approaches developed in this project across neurodevelopmental disorder trials. We need to further investigate the impact of minocycline in this project. We will continue to build the number of subjects in this project and determine the overall significance of the noted minocycline effects.

**Keywords:** Fragile X Syndrome, Minocycline, EEG

**Disclosure:** Nothing to disclose.

### T86. Single Dose Pharmacokinetics of Amphetamine Extended-Release Oral Suspension (AMPH EROS) in Children Aged 4 to 5 Years Old With Attention-Deficit/Hyperactivity Disorder

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**Background:** The primary objective of this pharmacokinetic (PK) study was to evaluate the concentration/time profile of amphetamine extended-release oral suspension (AMPH EROS, Dyanavel® XR; Tris Pharma, Inc., Monmouth Junction, NJ) in preschool children (ages 4 to 5 years) following a single 2.5 mg/mL dose of study drug as part of an FDA regulatory requirement.

The efficacy of AMPH EROS in the treatment of attention-deficit/hyperactivity disorder (ADHD) in children ages 6 to 12 years was established in a Phase 3, randomized, double-blind, placebo-controlled laboratory classroom study. In that study, ADHD symptoms in children on an optimized dose of AMPH EROS (range 10-20 mg/day) were statistically significantly improved compared with symptoms in children treated with placebo. For children treated with AMPH EROS, onset of effect was demonstrated at 1 h after dosing (the first time point studied), and efficacy was observed through 13 h post-dose. The effect size was

comparable to effect sizes demonstrated for other psychostimulants tested in studies using a similar design.

A previous PK study of AMPH EROS was conducted in children ages 6 to 12 years old diagnosed with ADHD, to evaluate the single-dose (10 mg) plasma amphetamine concentration/time profile of orally administered AMPH EROS. Following a single 10 mg (2.5 mg/mL) oral dose of AMPH EROS in 12 pediatric subjects under fasted conditions, d-amphetamine and l-amphetamine peak plasma concentrations occurred at a median time of 3.9 and 4.5 h after dosing, respectively. The mean plasma terminal elimination half-life of d-amphetamine was 10.43 (± 2.01) hours and the mean plasma terminal half-life for l-amphetamine was 12.14 (± 3.15) hours. These results were consistent with PK data reported for AMPH EROS in adults. Subsequently, a relative bioavailability study was conducted in 29 healthy adult subjects in a study utilizing a crossover design under fasted conditions. Following a single, 18.8 mg oral dose of AMPH EROS, the median (range) time to peak plasma concentration (tmax) for d- and l-amphetamine was 4.0 (2-7) hours after dosing and peak concentrations (Cmax) were 102% and 106% for d-amphetamine and l-amphetamine, respectively, when compared with the Cmax of the IR mixed amphetamine salts tablets. The relative bioavailability of AMPH EROS compared with an equal dose of mixed amphetamine salts IR tablets is 106% of d-amphetamine and 111% for l-amphetamine.

The data from the present study are intended to guide appropriate dosing for future safety and efficacy studies with AMPH EROS in the 4-5-year-old (preschool) patient population.

**Methods:** This open-label, single-dose, single-period, single-treatment study was designed to evaluate the PK profile of AMPH EROS in male and female subjects aged 4 to 5 years with weight ≥ 28 lbs at screening. Eligible subjects were diagnosed with ADHD by an appropriately-credentialed health care professional (psychiatrist, psychologist), using DSM-5 criteria and supported by a structured Kiddie-Schedule for Affective Disorders and Schizophrenia-Present and Lifetime Version (K-SADS-PL) interview administered at screening. All subjects provided written informed consent by both parents or legal guardians and verbal assent prior to administration of study procedures.

Demographics included descriptive statistics for age, sex, race, weight, and height. PK parameters for d- and l-amphetamine in plasma (Cmax, tmax, AUC0-t, AUC0-∞, and t1/2) were calculated and expressed as means, geometric means, and standard deviations.

The primary endpoint was all objective PK measurements at 28 h post-dose. Safety was monitored continuously and assessed based on occurrence of adverse events, as well as measurements of vital signs and ECG.

**Results:** Five (5) subjects (2 females and 3 males) completed the study. The mean age of enrolled subjects was 4 years old, with a mean (SD) BMI of 16.2 (1.1). Three subjects were African-American and 2 were Caucasian. For d-amphetamine, the mean (SD) Cmax, AUC0-t, and AUC0-∞ were 20.920 (2.292) ng/mL, 288.327 (48.096) hr*ng/mL, and 311.847 (46.287) hr*ng/mL, respectively. The median (range) tmax was 2.98 (2.97-3.98) hours and the mean (standard deviation) t1/2 was 6.81 (1.27) hours.

For l-amphetamine, the mean (SD) Cmax, AUC0-t, and AUC0-∞ were 6.550 (0.739) ng/mL, 96.481 (15.702) hr*ng/mL, and 106.842 (14.369) hr*ng/mL, respectively. The median (range) tmax was 3.98 (2.97-4.02) hours and the mean (standard deviation) t1/2 was 7.56 (1.56) hours.

Study drug was well-tolerated by the subjects in this study. No AEs, SAEs, or significant findings for vital signs or ECGs were reported.

**Conclusions:** The PK parameters for AMPH EROS in children 4-5 years old measured and assessed in this PK study were consistent with those observed in children aged 6 to 12 years and in adults.

**Keywords:** Pharmacokinetics, Children, ADHD

**Disclosure:** Tris Pharma, Employee

### T87. Nicotinic Driven Adolescent Shift of Local/Long-Range Input Balance Onto a Prefrontal Top-Down Projection Establishes Adult Attentional Control

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**Background:** Neuromodulation plays two separate essential roles at different ages: during early fetal age in cell development, and later in adulthood in altering neural dynamics and plasticity. Neuromodulatory tone is present in the brain between these two developmental stages, but its role during adolescence, when circuit formation occurs, is less extensively explored. The prefrontal cortex (PFC) experiences protracted development that extends through adolescence during which inputs from local PFC and distal brain regions are integrated into circuits that interface sensory and cognitive information to support complex cognitive behaviors. To what extent neuromodulatory mechanism contributes to this late developmental process is not known. Here, we examine the role of neuromodulation in circuit development of evolutionarily conserved PFC top-down neurons projecting from dorsal anterior cingulated cortex and secondary motor cortex to primary visual cortex (PFC- > VIS) in mice.

**Methods:** We use Bussey-Saksida TouchScreen-based 5-choice serial reaction time task (5CSRTT), which requires mice to sustain and divide attention among 5 brief light stimuli presented in random order on touchscreen, to assess attention performance, processing speed, and response control in male C57BL/6 mice (n = 6-9 mice). To knock-down and over-express Lynx1 selectively in PFC- > VIS projection neurons, we employed intersectional viral approach by injecting cre-dependent knock-down or over-expression virus of Lynx1 to PFC as well as retrograde-cre in VIS. Whole-cell patch clamp recordings, dendritic spine analysis, and rabies mediated monosynaptic input mapping were performed to assess connectivity onto top-down PFC- > VIS neurons.

**Results:** Rabies input mapping identified robust basal forebrain cholinergic inputs onto top-down PFC- > VIS projection neurons established by adolescence. However, electrophysiological recording reveals decreased nicotinic ACh response in adult PFC- > VIS projection neurons compared to adolescence, as the projection neurons undergo a shift in cell-autonomous suppression of nicotinic signaling through expression of a nicotinic brake, Lynx1 ($p < 0.05$). Bidirectional viral manipulations of Lynx1 expression within PFC- > VIS projection neurons revealed that adolescent, and not adult, Lynx1 expression is necessary and sufficient to develop adult attentional performance on the 5-choice serial reaction time task ($p < 0.05$). Exploration of Lynx1-dependent changes in connectivity onto PFC- > VIS projection neurons revealed that Lynx1 facilitates a selective reduction in heightened local connectivity onto top-down projections through the suppression of excessive dendritic spine formation that shifts the balance of local/long-range inputs in adulthood ($p < 0.05$).

**Conclusions:** Our study reveals that adolescent cell-autonomous molecular control over nicotinic neuromodulatory transmission is essential for prefrontal top-down projection neurons to shift the connectivity balance of local and long-range inputs to establish adult attentional control. These findings propose "local and long-range balance" as a key developmental milestone for cognitive development and offer a novel conceptual

framework for the better understanding of neurodevelopmental disorders and their therapeutic interventions.

**Keywords:** Prefrontal Cortex, Visual Cortex, Attention, Adolescence, Nicotinic Acetylcholine Receptors

**Disclosure:** Nothing to disclose.

### T88. Social Anxiety Severity and Peer Observation Heighten Error-Related Brain Responses in Adolescents

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**Background:** ERP research suggests that anxious youth show exaggerated neural responses to errors, compared to non-anxious youth. However, neuroimaging work does not consistently replicate this effect (e.g., Fitzgerald et al., 2013; Fitzgerald et al., 2018). Recent research suggests that this pattern may depend on the social context in which the task is completed (Barker et al., 2015; Buzzell et al., 2017). In particular, exaggerated neural response to errors in anxious youth was only present when they were being observed by a peer, and not when the participant completed the task alone. The present study extends these findings using fMRI to examine the joint impact of social anxiety severity and social context on error-related brain responses during adolescence.

**Methods:** In the current analysis, 20 youth (9-18 years old, $M = 13.75$, $SD = 2.66$) completed a modified version of the Erikson Flanker task during fMRI. Of the recruited participants, half met DSM-5 criteria for at least one anxiety disorder. All participants completed the Screen for Child Anxiety Related Disorders (SCARED) within 3 months of the imaging visit. The SCARED Social Anxiety subscale (averaged across child and parent reports) was used in all analyses to examine the impact of individual differences in social anxiety on task performance and brain response to errors. In the current sample Social Anxiety scores did not correlate with age ($r = -.05$, $p = .84$) or IQ ($r = .12$, $p = .67$) and were well-distributed across both anxious and control participants. Data collection is ongoing.

In the Flanker task, participants were instructed to press a button indicating which direction a center arrow, flanked by two arrows on either side, was pointing. Neuroimaging analyses focused on neural responses to errors on incongruent trials (when the flanking arrows faced the opposite direction of the center arrow). Participants completed 2, 6-minute runs of the task alone (alone condition) and 2, 6-minute runs while they believed they were being observed by a same-age, same-sex peer (peer condition). In the peer condition, participants were told that another participant was observing them during the task and would be making predictions about their task performance. In reality, there was no other participant observing and all communication between "participants" was pre-recorded (Smith, Chein, & Steinberg, 2014). Order of the social context manipulation was counterbalanced across participants. All neuroimaging data was analyzed using mixed-effects models-SCARED Social Anxiety scores were entered as the continuous, between-subject variable and social context (peer, alone) and task condition (incongruent error, incongruent correct) as repeated, within-subject variables. All analyses covaried for age.

**Results:** Whole brain analyses ($p > .005$, $k > 89$) revealed a significant social anxiety X social context X task condition interaction in orbitofrontal cortex (OFC), a region underlying calculations of reward valuation. Follow-up tests revealed that the participants with higher social anxiety demonstrated greater OFC engagement when processing errors when they believed they were being observed by a peer, compared to making an error when completing the task alone [$F(1,17) = 16.81$, $p = .001$; $\square p2 = .49$].

**Conclusions:** These findings demonstrate the joint impact of social anxiety severity and social context on neural responses to errors in adolescents. Future analyses should examine the relationship between error-related brain responses and subsequent task performance in order to begin to understand the impact of heightened OFC engagement on cognitive control processes. These findings highlight the need to consider the impact of social context on error processing across both clinical and sub-clinical levels of social anxiety.

**Keywords:** Social Anxiety, Adolescence, Functional Neuroimaging

**Disclosure:** Nothing to disclose.

### T89. Differential Roles of the Salience Network During Prediction Error Encoding and Facial Emotion Processing Among Female Adolescent Assault Victims

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**Background:** Dominant neurocircuitry models posit that the primary neural mechanisms mediating PTSD and related psychopathology following early life trauma include heightened emotion processing in the amygdala, dorsal anterior cingulate cortex (dACC), and anterior insula, with concurrent decreased emotion regulation / inhibition in medial prefrontal cortex (mPFC) and hippocampus. From a large-scale neural network perspective the amygdala, dACC, and anterior insula are individual nodes within a larger salience network, and hyperactivity of the individual nodes among trauma-exposed and PTSD samples has been conceptualized as reflecting hyperactivity of the larger salience network7.

While much research has investigated the neurocircuitry of emotion and threat, the impact of early life trauma on neural mechanisms of reinforcement learning has comparatively been under-investigated. This is striking, given that traumatic stress-related psychopathology is conceptualized from a learning perspective and a hallmark characteristic of PTSD is dysfunctional fear learning and fear extinction, processes that rely on reinforcement learning mechanisms.

The purpose of the current study was to directly test the hypothesis of differential dysfunction of the salience network during emotion processing versus reinforcement learning as a mechanism conferring risk following early life assaultive violence. We directly compared activity of the salience network (SN) during a facial emotion processing (FEP) task and during reinforcement learning (RL) tasks using either social or non-social stimuli among a sample of adolescent girls exposed to varying severities of assaultive violence.

**Methods:** Adolescent girls ($n = 30$ physically or sexually assaulted; $n = 30$ healthy comparison) aged 11-17 completed two tasks during fMRI: a facial emotion processing task and reinforcement learning (RL) tasks using either social or non-social stimuli. Independent component analysis was used to identify a large-scale salience network and characterize its engagement in response to emotion processing and prediction error (PE) encoding during the RL tasks. Participant's assaultive trauma histories were characterized via a structured interview.

**Results:** The higher-order assault exposure severity x task (RL versus FEP task) interaction compared PE encoding during both RL

tasks to facial emotion processing (FEP) collapsed across stimulus category and observed a significant assault x task interaction, t(327) = 3.56, p < .001 (Bonferroni corrected p = .007). This higher-order interaction was then decomposed by testing separate models for the RL and FEP tasks.

For the RL tasks, we observed a main effect of assault exposure severity indicating weakened SN encoding of negative PEs, t(99) = -3.50, p < .001, that was not moderated by social versus non-social RL task, t(99) = 1.16, p = .25. The relationship between weakened SN encoding of negative prediction errors and assault exposure severity remained when including PTSD symptom severity as a covariate, t(98) = -3.11, p = .002.

For the FEP task, there was a group x facial expression (neutral vs fear) x duration (covert vs overt) interaction, t(212) = 2.57, p = .011. This interaction was attributed to greater SN responses to overt fear faces in the highly assaulted compared to both other groups, t(106) = 2.09, p = .039 (Figure 2B). The group x facial expression x duration interaction remained significant when controlling for CAPS PTSD symptom severity, t(211) = 2.57, p = .011.

When examining the effect of PTSD symptom severity among the assaulted girls, the higher-order group x task interaction comparing both RL tasks to facial emotion processing (FEP) collapsed across stimulus category did not reveal any significant interaction or main effects of PTSD (all ps > .6).

**Conclusions:** Consistent with dominant models, the results demonstrated that SN activity during emotion processing increased with the severity of assaultive violence exposure. By contrast, SN encoding of negative prediction errors decreased with the severity of assault exposure. This differential response of the SN has implications for our understanding of the neural mechanisms by which early life trauma confers risk for PTSD and other mood and anxiety disorders.

The data suggest that SN hyperactivity in a particular cognitive-affective domain among early life trauma victims (e.g., facial emotion processing) should not necessarily be expected to generalize to other cognitive-affect domains (e.g., negative PE encoding), which is consistent with dissociable mechanisms in the SN and distinct patterns of SN alterations among trauma-exposed youth. This pattern of data suggests the utility of SN models of early life trauma exposure may come from further careful delineation of the SN activity across clinically-relevant cognitive domains.

In this sample of youth who directly experienced violence inflicted upon them by another person, weakened SN responsivity towards unexpectedly negative social behavior could reflect learned blunted responding that develops as an adaption following toxic early social environments and helps maintain learned social associations. Weakened anterior insula has also been linked to decreased detection of untrustworthiness and thereby possibly suggests a mechanism explaining heightened risk of revictimization among youth exposed to early life trauma.

**Keywords:** Childhood Trauma, Salience Network, Functional Neuroimaging, Reinforcement Learning, Emotion

**Disclosure:** Nothing to disclose.

### T90. A Haplotype of the Norepinephrine Transporter Gene Modulates Intrinsic Brain Activity, Visual Memory, and Visual Attention in Children With Attention-Deficit/Hyperactivity Disorder

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**Background:** The norepinephrine transporter gene (SLC6A2) and deficits in visual memory and visual attention have been consistently reported to be associated with attention-deficit/hyperactivity disorder (ADHD). Despite the knowledge of SLC6A2 functionality related to ADHD, only a few studies have investigated the effects of genetic variations in SLC6A2 on the brain in patients with ADHD. This study examined whether the SLC6A2 rs36011 (T)/rs1566652 (G) haplotype affected intrinsic brain activity in children with ADHD and whether those alterations were associated with visual memory and visual attention.

**Methods:** A total of 96 drug-naïve children with ADHD and 114 typically developing children (TDC) were recruited. Visual memory and visual attention were assessed by Delayed Matching to Sample (DMS) and Rapid Visual Information Processing (RVP) tasks, respectively. The SNP genotyping of rs36011 and rs1566652 was performed by the method of matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS). Resting-state (RS) EPI preprocessing, including slice timing, realignment, within-participant registration of RS-fMRI data and T1 images, and spatial normalization, was performed using Data Processing Assistant for RS-fMRI (DPARSF) toolbox based on Statistical Parametric Mapping (SPM8, Wellcome Trust Centre for Neuroimaging, London, UK). We analyzed voxel-wise intrinsic brain activity with regional homogeneity (ReHo) and degree centrality (DC). The effects of diagnosis, haplotype, and the diagnosis-by-haplotype interaction on the ReHo and DC were examined. To reduce type I error, all significant clusters in neuroimaging-related statistical analyses were corrected for multiple comparisons at the cluster level by controlling topological family-wise error (FWE) calculated based on Gaussian Random Field theory, using a cluster-forming voxel-level height threshold of $p < 0.005$ and a spatial extent threshold that ensured a cluster-wise FWE at $p < 0.005$. We also examined the correlations between visual memory, visual attention, and SLC6A2-modulated intrinsic brain activity.

**Results:** These 210 participants were classified as either carriers (n = 102) of the SLC6A2 rs36011 (T)/rs1566652 (G) haplotype (45 ADHD-TG, 57 TDC-TG) or non-carriers (n = 108) of this TG haplotype (51 ADHD-Non-TG, 57 TDC-Non-TG). Compared with TDC, children with ADHD showed decreased ReHo in bilateral cuneus and lingual gyri and decreased DC in the left cuneus and lingual gyrus. Compared with the noncarriers, the TG carriers showed significantly increased DC in the right precentral and postcentral gyri. Children with ADHD-TG had significantly increased ReHo and DC in bilateral precentral and postcentral gyri than their Non-TG counterparts. Significant diagnosis by TG haplotype interactions was found in the right postcentral gyrus and superior parietal lobule for ReHo. For the ADHD group, ReHo in the right postcentral gyrus and superior parietal lobule increased with the TG haplotype; whereas the TDC group showed the reverse pattern. For the ADHD-TG group, there were positive correlations of mean latency of correct responses in simultaneous tasks in DMS with the ReHo of bilateral precentral-postcentral gyri; positive correlations of mean latency of correct responses in simultaneous and delay tasks in DMS with the ReHo of the right postcentral gyrus-superior parietal lobule; negative correlations of total hits in RVP with the DC of bilateral precentral-postcentral gyri; and positive correlations of total misses in RVP with bilateral precentral-postcentral gyri.

**Conclusions:** Our work is the first study to demonstrate that ReHo and DC in sensorimotor and dorsal attention networks are affected by the SLC6A2 rs36011 (T)/rs1566652 (G) haplotype in children with ADHD, and also the first to demonstrate that these SLC6A2-modulated alterations in ReHo and DC are related to visual memory and visual attention in ADHD. A novel gene-brain-behavior association was identified in which the intrinsic brain activity of the sensorimotor and dorsal attention networks was related to visual memory and visual attention in ADHD children

with the SLC6A2 rs36011 (T)/rs1566652 (G)haplotype. Future prospective studies are needed to clarify the relationship between developmental trajectories of intrinsic brain activity and SLC6A2 genotype in children with ADHD.

**Keywords:** Norepinephrine Transporter Gene (SLC6A2), Attention-Deficit/Hyperactivity Disorder, Resting-state fMRI, Visual Memory, Visual Attention

**Disclosure:** Nothing to disclose.

### T91. Neurofunctional Development of Aversive Face Processing During Puberty: Changes in Fronto-Amygdalar Effective Connectivity

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**Background:** Puberty is a time of increased onset and the emergence of sex differences in several neuropsychiatric diseases. Neural processing of faces is reported to be altered in some of these disorders. Brain regions that are important for face and emotional processing, such as the medial prefrontal cortex (mPFC) and the amygdala, undergo substantial neurodevelopment during adolescence and into adulthood. However, no study has yet examined the impact of pubertal status on such findings. Here, we measure brain function associated with face processing in pre- and post-pubertal children as defined by clinician-rated pubertal stage, as well as in healthy adults, to determine the impact of development on affective face processing.

**Methods:** One-hundred and seventeen participants were categorized into three developmental groups: prepubertal, pubertal, and adult. Assignment to prepubertal or pubertal groups was made according to a clinician-rated pubertal stage (PS) scale that is based on the development of breast tissue in girls and testicular volume in boys. Functional MRI (fMRI) data were collected on a 3 T GE scanner to measure blood-oxygen-level dependent (BOLD) activation during a face processing task. The experimental paradigm consisted of a picture-matching task with five conditions: faces or scenes with aversive (angry/sad/disgusted/fearful/surprised) or nonaversive (happy/neutral) valence as well as sensorimotor control images (scrambled images). The scrambled images were subtracted from all face/scene stimuli. Data were pre-processed with SPM5, and then warped to a study-specific MNI template using ANTS, spatially smoothed (8 mm), and motion-corrected using ART ( < 0.5 mm frame-wise displacement). Next, data were analyzed in AFNI using a multivariate model to identify voxel-wise main and interactional effects of image type (faces/scenes) and valence (aversive/nonaversive) on BOLD signal while controlling for sex and pubertal status. For all regions that were identified as specifically responsive to aversive faces in the voxel-wise analysis, we assessed the impact of both sex and developmental group on effective connectivity measured with psychophysiological interactions (PPI) in SPM5.

**Results:** There were 58 prepubertal children (PS1, $8.7 \pm 0.3$ years, 41.3% girls), 31 pubertal adolescents (PS2-5, $13.0 \pm 0.7$ years, 45.2% girls), and 28 adults ($36.1 \pm 6.9$ years, 53.6% women). The voxel-wise analysis applying the multivariate model demonstrated several regions that were specifically responsive to aversive faces ($p < 0.005$, uncorrected). These included the left and right amygdala, which were used as seeds in the subsequent PPI analyses. Our data showed a main effect of developmental group on effective connectivity of both the right and left amygdala with the ventral medial PFC (Brodmann Area 11, [BA11]) bilaterally ($p < .005$, uncorrected). Whereas effective connectivity was positive in the prepubertal children and the adults, the functional relationship was negative in pubertal adolescents. In addition, connectivity of the right amygdala with left BA25 was similarly reduced in the pubertal cohort ($p < .005$, uncorrected) compared to prepubertal children and adults. Within the pubertal group, there was a trend for boys to have more robustly negative effective connectivity than girls of right amygdala to left BA25 as well as to left BA11 ($p's < .10$). PPI analyses also showed a main effect of sex on right and left amygdala effective connectivity with several clusters ($p < .005$, uncorrected), including the superior frontal gyrus (BA6) for the right amygdala and the left parietal cortex (BA31, BA40) and right temporal parietal junction (BA39) for the left amygdala. In all these functional relationships, females showed more robust positive effective connectivity. Finally, there were no regions identified in the interaction between developmental group and sex.

**Conclusions:** These data demonstrate that during puberty, effective connectivity in response to viewing aversive faces is altered, with a switch from positive to negative connectivity of the amygdala with orbitofrontal and subgenual PFC. Previous studies spanning childhood to early adulthood have demonstrated similar age-related changes (i.e., from positive to negative fronto-amygdalar effective connectivity) in response to aversive faces. Our data also suggest that onset of puberty is associated with the changeover to negative amygdala connectivity, although age and pubertal status cannot be disentangled in the current sample. Further, sex differences in negative amygdala connectivity may emerge during puberty (boys tended to have stronger negative connections) and reverse in adulthood. Our findings of altered processing of aversive faces during puberty may provide neurobiological insight into the increased vulnerability for the onset of psychopathology that is observed at this time.

**Keywords:** Neurodevelopment, Face Emotion Processing, fMRI Effective Connectivity, PPI, Affective Neuroscience

**Disclosure:** Nothing to disclose.

### T92. Altered Functional Connectivity Across Large-Scale Brain Networks Predicts Attentional Control Deficits and Response to Cognitive-Behavioral Therapy in Pediatric Obsessive-Compulsive Disorder

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**Background:** Obsessive-compulsive disorder (OCD) is a disabling neuropsychiatric disorder characterized by intrusive thoughts (obsessions) and repetitive, ritualistic behaviors (compulsions). Neuroimaging studies of OCD have consistently identified structural and functional abnormalities in corticostriatal brain networks. Emerging evidence from investigations of large-scale brain networks (e.g., default-mode, salience, ventral and dorsal attention, cingulo-opercular, and frontoparietal) suggest that more diffuse imbalances in brain connectivity may underlie the pathophysiology of OCD. Few studies have assessed connectivity within or between these networks in pediatric OCD or how patterns of connectivity are associated with cognitive processes noted to be dysfunctional in OCD. None have assessed how such patterns associate with treatment response in pediatric OCD.

**Methods:** Resting state fMRI (rsfMRI) scans and behavioral data from the Continuous Performance Task (CPT) were acquired from 25 pediatric OCD patients (12.8 + /- 2.9 years) and 20 age- and sex-matched healthy controls (HC; 11.0 + /- 3.3 years). Twenty-two of the OCD patients completed a 16-week Cognitive Behavioral Therapy (CBT) intervention for OCD. OCD symptoms were assessed at baseline and end of treatment using the Children's Yale-Brown Obsessive-Compulsive Scale (CY-BOCS). Functional connectivity was assessed by sampling the entire brain using 352 cortical nodes defined based on the Cortical Area Parcellation from Resting-State Correlations dataset (Gordon et al., 2016) and 19 additional subcortical and cerebellar areas defined based on the Freesurfer software. For each participant, pairwise correlation coefficients of rsfMRI time-series data were computed between each node. Analyses examining group differences in the resulting functional connectivity matrix were conducted and corrected for multiple comparisons using network-based statistic (NBS) in the NBS Toolbox (Zalesky et al., 2010), specifically using a statistical threshold $p = .05$, 10 000 permutations, and t-threshold = 4. For each significant network component, the average connectivity strength across significant edges was calculated and used in secondary analyses conducted in IBM SPSS (v23). Multivariate analyses were used to further examine and control for the effects of age, sex, and head motion (i.e., mean framewise displacement) on group differences in connectivity. In the OCD group, paired t-tests were used to assess treatment response and linear regression was used to test relationships between baseline connectivity strength and changes in OCD symptoms. Finally, independent t-tests were used to examine group differences in CPT performance (commission errors and reaction times) and univariate analyses were used to explore relationships between connectivity and CPT performance across groups.

**Results:** Significantly reduced connectivity in OCD relative to HC participants was detected in two network components, both connecting regions from the default mode network (DMN) with regions from task positive networks (i.e., salience, ventral and dorsal attention, cingulo-opercular, and frontoparietal). No significant effects of age, sex and motion on connectivity were detected ($ps > .1$) and group differences remained significant after controlling for these variables ($ps < .001$). In the OCD group, CY-BOCS scores decreased pre-to-post CBT ($ps < .001$). Average connectivity in the first network component correlated negatively with symptom improvement ($p = .030$). On the CPT, OCD patients responded significantly faster and made more commission errors than HC participants. For both network components, weaker connectivity was associated with faster responses and more errors ($ps < .05$) across both groups (no group interaction, $ps > .1$).

**Conclusions:** These findings point to an imbalance between task positive and task negative networks in pediatric OCD that may underlie deficits in attentional control. Such imbalance may also contribute to the impaired control over intrusive thoughts early in the illness. This is the first study to show that altered connectivity across these large-scale networks may predict response to CBT in pediatric OCD. Follow-up fMRI data from this sample are currently being analyzed to examine changes in connectivity that coincide with changes in OCD symptoms following CBT. These preliminary findings set the stage for the development of novel strategies to target these networks in the service of improving attentional control and preventing the development or worsening of OCD symptoms.

**Keywords:** Resting State Functional Connectivity, Obsessive-Compulsive Disorder (OCD), Pediatric, Cognitive Behavior Therapy, Attention

**Disclosure:** Nothing to disclose.

## T93. Development of a Coding System for Quantifying Simple Interactions Between Adults and Young Children

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**Background:** Supportive relationships with caring adults are among the most important environmental influences on children's brain development. Children growing up in adverse circumstances are at higher risk for a vast number of negative behavioral, health and mental health outcomes throughout their lifespan. However, evidence suggests that improving the quality of interactions between adults and young children can buffer the impact of this adversity. Thus, researchers need a reliable, quantitative means of assessing adult-child interactions that captures the behaviors that are known to improve child development.

**Methods:** For this study, 48 videos (9.11 ± 0.25 minutes each) of 3-6-year-old children and a parent playing together as a parent-child dyad were collected and coded using The Observer software (Noldus, The Netherlands). We adapted the Simple Interactions qualitative behavioral coding system (SI Tool), originally designed score interactions using one score for each video, to instead code interactions on a moment-by-moment basis. The coding system scores the behavioral dimensions of Connection, Reciprocity (also known as 'serve and return') and Opportunity to Grow. This moment-by-moment coding system provided a means of quantifying the percent time spent in each level of each behavioral dimension.

**Results:** Using this new coding strategy, intraclass correlation coefficients for the behavioral dimensions of Connection, Reciprocity, and Opportunity to Grow were 0.93, 0.95, and 0.93, respectively. To assess the construct validity of this coding strategy, one scorer with significant experience coding videos using the standard SI Tool methodology scored 21 of the 45 videos. Correlation between the standard SI Tool scoring and moment-by-moment scoring was highly significant ($rho = 0.74$, $p < 0.001$).

**Conclusions:** This new quantitative scoring system provides a reliable way to assess the percentage of time an adult-child dyad spends interacting with low, moderate, or high quality of Connection, Reciprocity, and Opportunity to Grow, providing a quantitative assessment of interaction quality. This quantitative coding system has high validity with traditional qualitative Simple Interactions coding and a high level of inter-rater reliability providing a very useful tool for studying child-adult interactions in research studies. This new quantitative coding system will allow researchers to rigorously study how adult-child interactions affect child development, as well as buffer children against the impact of stress.

**Keywords:** Adult-Child Interactions, Reciprocity, Connection, Serve and Return

**Disclosure:** Nothing to disclose.

## T94. Autism Risk After Prenatal Exposure to Medication Affecting Neurotransmitter Systems

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**Background:** Prenatal exposure to certain medications has been hypothesized to influence the risk of autism spectrum disorder (ASD). However, the underlying effects on the neurotransmitter systems have not been comprehensively assessed. This study investigates the effects of early-life interference with different neurotransmitter systems, by medication, on the risk of ASD in offspring.

**Methods:** Using data from an Israeli national health provider, this was a case-cohort study of children born between 1997 and 2007 and followed up for ASD until January 2016. We estimated hazard ratios (HRs) and 95% confidence intervals (CIs) of ASD in children prenatally exposed to medication affecting neurotransmitter systems, compared to non-exposed children. Using publicly available data, we identified 55 groups of such medications prescribed to pregnant women in our sample. We investigated the effects of exposure to these medication groups using Cox proportional hazard regression, adjusting for the relevant confounders (birth year, maternal age, maternal history of psychiatric and neurological disorders, maternal number of all medical diagnoses a year prior to pregnancy).

**Results:** The analytical sample included 95,978 individuals (mean [SD] age at the end of follow-up 11.6 [3.1] years; 48.8% female; 1,405 cases with ASD, 94,573 controls). We tested 34 groups of medications, 5 of which showed statistically significant ($p < 0.05$) association with ASD in fully adjusted models. There was also an evidence of confounding effects of the number of maternal diagnoses on the association between offspring exposure to medication and ASD. Adjusting for this factor, we observed lower estimates of ASD risk among children exposed to cannabinoid receptor agonists (HR = 0.72 (0.55-0.95), $p = 0.02$), muscarinic receptor 2 agonists (HR = 0.49 (0.24-0.98), $p < 0.05$), opioid receptor κ and ε agonists (HR = 0.67 (0.45-0.99), $p < 0.05$), or adrenergic receptor α 2 C agonists (HR = 0.43 (0.19-0.96), $p = 0.04$). Exposure to antagonists of neuronal nicotinic receptor α was associated with higher estimates of ASD risk (HR = 12.94 (1.35-124.25); $p = 0.03$).

**Conclusions:** In this sample the majority of medications affecting neurotransmitter systems had no effect on the estimates of ASD risk. Results require replication and/or validation using experimental techniques.

**Keywords:** Autism, Pharmacoepidemiology, Risk, Exposure, Maternal

**Disclosure:** Nothing to disclose.

### T95. C-Reactive Protein and Response to Lurasidone Treatment in Children and Adolescents With Bipolar Depression

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**Background:** C-reactive protein (CRP), an acute phase reactant, is a non-specific but sensitive marker of systemic inflammation. Several studies have suggested that inflammation may play a significant role in the development of depressive symptoms and cognitive impairment in various forms of psychiatric illness. In a short-term clinical trial of adults with bipolar depression randomized to receive flexibly dosed lurasidone (20-120 mg/d) or placebo, lurasidone treated patients with high baseline CRP levels demonstrated a larger treatment effect compared to patients with lower baseline CRP levels (Raison et al. et al., 2018). The current analysis explores the association between CRP levels, depressive symptoms, and cognition in a short-term, placebo-controlled clinical study of lurasidone in children and adolescents with bipolar depression (DeBello et al., 2018).

**Methods:** Patients 10 to 17 years of age with a DSM-IV-TR diagnosis of bipolar I depression, were randomized to 6 weeks of double-blind treatment with once-daily, flexible doses of lurasidone (20-80 mg) or placebo. The primary efficacy outcome measure was the change from baseline to week 6 in the Children's Depression Rating Scale, Revised (CDRS-R). Treatment response was defined as 50% or greater improvement on the CDRS-R from baseline to week 6. Cognitive function was evaluated as a safety measure with the computerized Brief Cogstate Battery at baseline and week 6.

A high-sensitivity CRP (hsCRP) assay was used to assess levels of systemic inflammation. HsCRP was evaluated as a logarithmically transformed continuous variable and as a categorical variable dichotomized into lower ( < 1 mg/L) and higher ( > 1 mg/L) subgroups. Because body mass index (BMI) is associated with CRP levels (O'Connor et al., 2009), baseline BMI, as well as age, gender and race were adjusted in the analysis. The percentiles for the BMI categories were derived based on the WHO 2007 growth reference for 5 to 19 years old. Statistical interaction tests were applied to evaluate whether baseline hsCRP is associated with differential response to lurasidone treatment (vs. placebo) on measures of depressive symptoms and cognitive function in children and adolescents with bipolar depression.

**Results:** A total of 248 patients (74%) had a baseline hsCRP serum concentration < 1 mg/L. The statistical interaction between stratified baseline hsCRP ( < 1 mg/L vs. > 1 mg/L) and lurasidone (vs. placebo) was significant for changes in CDRS-R score from baseline to week 6 ($p < 0.05$, $p = 0.08$ for logCRP-treatment interaction), with a larger placebo-corrected effect sizes for lurasidone in patients with higher baseline serum concentrations of hsCRP ( > 1 mg/L). Analysis by baseline BMI showed a similar lurasidone treatment response (vs. placebo) in underweight/normal BMI patients (NNT = 4.5, BMI < 85th percentile based on WHO BMI status categories) and overweight/obese patients (NNT = 4.3, BMI > 85th percentile) but only for patients with baseline hsCRP levels < 1 mg/L. Among patients with higher baseline CRP levels, there was a significantly greater treatment effect in the underweight/normal subgroup (NNT = 1.8) compared to the overweight/obese subgroup (NNT = 5.2) (BMI by stratified CRP-treatment interaction $p < 0.05$, adjusted for age, gender, and race).

Analysis of cognitive function as assessed by Cogstate composite z-score showed consistent treatment benefit of lurasidone over placebo in the overweight/obese subgroup regardless of baseline CRP level. However, in the normal/underweight subgroup, only higher baseline CRP levels were associated with improvement in cognitive function for lurasidone treated patients compared to placebo.

As in previous studies, lurasidone was not associated with a significant effect on hsCRP level during the study.

**Conclusions:** In this post hoc analysis, baseline levels of the non-specific inflammatory marker CRP were associated with improvement in depressive symptoms and cognitive function among pediatric patients with bipolar depression treated with lurasidone. Consistent with previous findings in adults with bipolar depression, a larger lurasidone treatment effect was observed in children and adolescents with higher levels of CRP at study baseline. Stratification by baseline BMI suggests that bodyweight may moderate the relationship between CRP and the therapeutic response to lurasidone (vs. placebo).

**Keywords:** C-Reactive Protein, Lurasidone, Bipolar Depression, Children and Adolescents

**Disclosure:** Sunovion Pharmaceuticals, Inc, Employee

## T96. Glycine Transporter Inhibition Improves Conspecific-Provoked Immobility of the Balb/c Mouse Model of Autism Spectrum Disorder, an Effect Unrelated to the Corticosterone Response

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**Background:** Balb/c mice display impaired sociability and serve as a model of autism spectrum disorder (ASD). Relative to Swiss Webster (SW) mice, Balb/c mice have decreased locomotor activity in the presence of stimulus mice and spend less time exploring enclosed stimulus mice, whereas their exploratory behavior toward an inanimate object does not differ. Moreover, relative to the SW comparator strain, Balb/c mice display fewer discrete episodes of social approach, anogenital sniffing and mounting toward the ICR social stimulus mouse, among other deficient prosocial behaviors. Interestingly, Balb/c and SW mice do not differ in their number of open-arm entries in the elevated-plus maze. D-Cycloserine, a partial glycineB agonist, and VU0410120, a novel glycine type 1 transporter (GlyT1) inhibitor, improved the sociability of Balb/c mice, consistent with data suggesting that NMDA receptor (NMDAR) activation regulates sociability, and the endogenous tone of NMDAR-mediated neurotransmission is altered in this strain. The present study explored differences in conspecific-provoked immobility between the Balb/c and SW mouse strains, and the relationship between conspecific-provoked immobility and the corticosterone (CORT) response, a measure of acute stress, in these strains. Moreover, the effect of a prosocial dose of VU0410120 on the conspecific-provoked immobility and CORT response of the Balb/c mouse was also explored.

**Methods:** A standard 3-chamber apparatus was used to assess sociability. Experimentally-naïve, 4-week old male, outbred SW and genetically-inbred Balb/c test mice ($N \geq 21$ per condition) were individually weighed prior to drug/vehicle administration. Stimulus mice were 4-week old male ICR mice. VU0410120 dissolved in 20% HP-β-cyclodextrin or 20% HP- β-cyclodextrin alone (vehicle) was injected ip in a volume of 0.01 ml/g of body weight. Behavioral testing was performed 20 min after injection. Immobility was measured (discrete episodes, total seconds/10 min session, and seconds/discrete episode of immobility) in sessions II (enclosed stimulus mouse) and III (freely-behaving stimulus mouse) and defined as stationary while not engaged in social/stereotypic behavior. Immediately following behavioral testing, mice were sacrificed, and trunk blood was collected. Serum CORT levels were measured in duplicate using ELISA (ab108821) and only mean values with CVs below 12 were used in data analysis.

**Results:** Balb/c mice showed no significant relationship between transitions during session I and immobility in sessions II and III. On all measures, Balb/c mice were significantly more immobile than SW mice in sessions II and III. There was no significant difference in serum CORT levels drawn ≤ 30 min after the end of session III between vehicle-treated Balb/c and SW mice. CORT levels did not differ between groups of vehicle-treated Balb/c mice with high ($N = 8$) or low ($N = 9$) immobility scores (total seconds) in session III. Low and high immobility of the Balb/c strain in session III was based on the immobility of the Swiss strain in the vehicle condition: low immobility < 73 sec, and high immobility > 73 sec in a 600 sec epoch. Balb/c mice ($N = 21$) treated with VU0410120 (18 mg/kg) showed significantly lower scores of immobility and higher CORT levels compared to vehicle-treated mice. However, the CORT levels of VU0410120-treated Balb/c mice ($N = 11$) with the highest ($N = 3$) and lowest ($N = 8$) immobility scores (total seconds) in session III did not differ between groups.

**Conclusions:** Because high levels of comorbid anxiety are often seen in children with ASD, we explored serum CORT levels and immobility while Balb/c and SW test mice socially interacted with ICR stimulus mice. No relationship was found between locomotor activity of Balb/c mice in the absence of a social stimulus and their immobility in the presence of enclosed or freely-behaving stimulus mice. Balb/c and SW mice differed in terms of their immobility in the presence of enclosed and freely-behaving stimulus mice; moreover, the conspecific-provoked corticosterone (CORT) response did not mirror immobility behavior. A prosocial dose of VU0410120 significantly reduced the conspecific-provoked immobility of Balb/c mice and significantly increased the conspecific-provoked CORT response, compared to the vehicle-treated condition. However, the intensity of conspecific-provoked immobility in the VU0410120-treated Balb/c mice did not differ as a function of their CORT response. Thus, the "anxiolytic" effect of VU0410120 did not appear to be related to the conspecific-provoked CORT response in the Balb/c strain. Thus, in Balb/c mice, the stressfulness of a social encounter alone may not be the sole determinant of their increased immobility; perhaps, these mice also display an element of social "disinterest".

**Keywords:** Balb/c Mouse, ASD, Psychosocial Stress, Immobility, Corticosterone

**Disclosure:** Nothing to disclose.

## T97. Cyfip1 Haploinsufficiency Increases Compulsive-Like Behavior and Modulates Palatable Food Intake: Implications for Prader-Willi Syndrome

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**Background:** Binge eating (BE) is a heritable trait associated with eating disorders and involves rapid consumption of a large quantity of food. The genetic factors underlying BE are not yet known. We identified cytoplasmic FMRP-interacting protein 2 (Cyfip2) as a major genetic factor underlying BE and concomitant compulsive-like behaviors in mice. CYFIP2 is a gene homolog of CYFIP1 - one of four paternally-deleted genes in patients with the more severe Type I Prader-Willi Syndrome (PWS). PWS is a neurodevelopmental disorder where 70% of cases involve paternal deletion of 15q11-q13. PWS symptoms include hyperphagia, obesity (if untreated), cognitive deficits, and obsessive-compulsive behaviors. We tested whether Cyfip1 haploinsufficiency (Cyfip1 + /-) would enhance premorbid compulsive-like behavior (marble burying, hole board) and palatable food (PF) intake in a parent-of-origin-selective manner in both female and male mice on two different Cyfip2 genetic backgrounds.

**Methods:** Our initial studies involved Cyfip1 + /- mice on the BE-prone C57BL/6 N (B6NJ) background that possess the BE-associated missense mutation in Cyfip2, we tested the effect of Cyfip1 haploinsufficiency on compulsive-like and BE of sweetened PF on a B6NJ background. Thus, we also tested Cyfip1 + /- mice on a mixed C57BL/6 J (B6J)/B6NJ background where we backcrossed mice to homozygosity for the wild-type B6J allele at Cyfip2. We reasoned that detection of the hypothesized increased binge eating in Cyfip1 + /- mice would be facilitated on an initially low BE background and minimize potential ceiling effects associated with the Cyfip2N/N mutation.

**Results:** Cyfip1 + /- increased compulsive-like marble burying behavior on both backgrounds and also increased hole board

behavior on the Cyfip2J/J background. Furthermore, Cyfip1 + /- increased PF consumption in B6NJ mice in a paternally-enhanced manner. Contrary to our expectations, Cyfip1 + /- decreased PF consumption on the Cyfip2J/J background. This result was entirely driven by the effect of maternal Cyfip1 deletion in male mice where, surprisingly, this mode of inheritance induced a robust induction of binge eating in male wild-type mice. Finally, paternal Cyfip1 + /- on the same Cyfip2J/J background dramatically increased initial PF intake in females while it had no effect in males. In examining regulation of Cyfip1 expression on the Cyfip2J/J background, in the hypothalamus, there was a maternally-enhanced reduction of Cyfip1 transcription, but a paternally-enhanced reduction of CYFIP1 protein. In the nucleus accumbens, there was a maternally enhanced reduction of CYFIP1 protein which was in line with the enhanced effect of maternal deletion on eating behavior on the Cyfip2J/J background.

**Conclusions:** To summarize, increased compulsive-like behavior, parental origin-, and genetic background-dependent effects of Cyfip1 haploinsufficiency on PF consumption implicate a contribution of CYFIP1 to behaviors in neurodevelopmental disorders involving reduced expression of CYFIP1, including PWS, Fragile X Syndrome, and 15q11.2 Microdeletion Syndrome. More generally, these results not only highlight the importance of testing the behavioral and molecular effects of gene knockout in both sexes and on multiple genetic backgrounds, but also the importance of either controlling for or explicitly powering studies to examine parent-of-origin effects of haploinsufficiency on phenotypes of interest. Future studies could test the importance of Cyfip1 + /- in combination with other genes strongly implicated in PWS, including Magel2 and Snord116.

**Keywords:** OCD, Binge Eating Disorder, Food Addiction, Neurodevelopmental Disorders, Overeating

**Disclosure:** Nothing to disclose.

### T98. Prediction of BMI Changes in Adolescent Anorexia Nervosa After Intensive Treatment, Using Resting-State Functional MRI and Machine Learning

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**Background:** Anorexia Nervosa (AN) is an often difficult-to-treat psychiatric disorder. It has the highest mortality rate among all psychiatric disorders and high relapse rates (35-41% after 18 months). Low body mass index (BMI) is a characteristic feature of AN and return to abnormally low BMI after treatment is a particularly perilous outcome given the associated medical morbidity. To date, although multiple neuroimaging studies have provided mechanistic insights into AN, there is limited literature on how neural processes at a given time might relate to, or predict, clinical outcomes such as BMI at a future point. Resting-state functional MRI (rs-fMRI) is a neuroimaging measure that potentially carries important information regarding not only the pathophysiology of psychiatric conditions but also the propensity for wellness and maintaining improvements. In this study, we used rs-fMRI connectivity features measured after intensive treatment to predict changes in BMI values 3 months later. Similar approaches have been successful in predicting clinical outcome in other psychiatric disorders such as obsessive-compulsive disorder.

**Methods:** Our study included weight-restored and partially weight-restored adolescent females with AN (N = 16, aged 13-18), and matched healthy controls (HC, N = 17, aged 14-19) who were enrolled and scanned at the end of an intensive treatment program. Rs-fMRI data was obtained in a 3 T Siemens Prisma MRI scanner (TR/TE = 720/37 ms, flip angle = 52 degrees, voxel size = 2 mm isotropic, 64 channel head coil). Procedures were approved by UCLA IRB, and informed consent was obtained. Participants were instructed to keep their eyes open while viewing a white cross on a dark background, and not engage in specific thoughts or tasks. BMI values were measured on scan-day, as well as 3 months after. The difference in BMI percentile (age-adjusted) scores between the two measurements (BMI3-0) was the predicted variable.

Data were pre-processed in the CONN software, based on SPM12, with the following steps: brain extraction, motion correction, normalization to MNI space, and regressing out white-matter, CSF, motion and global signals. This was followed by hemodynamic deconvolution to minimize the confound of HRF variability, and band-pass filtering (0.01-0.1 Hz). Mean regions-of-interest (ROI) time series were extracted from a 390-region whole-brain atlas comprised of the HCP cortical atlas, the Harvard-Oxford subcortical atlas, and the Buckner cerebellar atlas. Functional connectivity (FC) was computed between all pairs of time series using Pearson's correlation. Additionally, we computed graph network measures using FC (clustering coefficient measuring functional segregation and shortest path length measuring functional integration), with the rationale that it carries unique information about overall network topology that is not available through FC.

Next, to identify FC features for the prediction analysis, we utilized a recursive feature elimination-based support vector machine classifier to classify AN individuals from HCs. This identified features that could best classify AN vs HC with high accuracy, which were then used in further steps. We first performed partial least squares (PLS) regression to explore the aggregate associative relationship between imaging features and BMI3-0 values, for assessing variance explained in BMI3-0 with all imaging features taken together. Next, we performed support vector regression (SVR) to predict BMI3-0 values. The latter step performed prediction at the single-subject level, while the former step merely provided their associative statistical relationship. Participants were randomly split in half, with one-half being used to develop the regression model, which was tested on the other independent half to make a prediction of their BMI3-0 values. This process was repeated over one million iterations, with participants randomly split in each iteration. Finally, the association between predicted and actual BMI3-0 values was computed. This procedure was performed independently with connectivity features, as well as with a feature set comprised of both connectivity and graph measures.

**Results:** Connectivity values, taken together, explained 50.6% variance in BMI3-0 (P = 0.002). Top predictive features were associated with the fronto-parietal task control and salience networks, along with connections from ventral visual stream and orbito-frontal cortex. The variance explained increased to 65.4% (P = 0.00015) when graph measures were included as features. Functional integration between DLPFC and anterior insula, and fronto-parietal FC explained maximum share of this variance.

With machine learning prediction, we found that predicted and actual BMI3-0 values were significantly associated when connectivity features were used (R = 0.54 ± 0.1, R^2 = 0.32 ± 0.1, P = 0.02 ± 0.01), and the prediction performance improved when graph measures were included in the feature set (R = 0.72 ± 0.05, R^2 = 0.53 ± 0.07, P = 0.001 ± 0.0006).

**Conclusions:** Our algorithm could significantly predict change in BMI 3 months later. Prognostic performance was superior when connectivity and graph measures were used together. Taken together, rs-fMRI displays promise as a tool to predict clinical outcomes in AN, yet results need to be replicated in a larger sample. If replicated, such an algorithm could help create

individualized treatment plans that could potentially reduce relapse rates in this population.

**Keywords:** Anorexia Nervosa, Body Mass Index, Resting State Functional Connectivity, Machine learning, Computational Psychiatry

**Disclosure:** Nothing to disclose.

### T99. Soluble Epoxide Hydrolase Activity and Protein in White and Asian Women With Anorexia Nervosa – a Proof-Of-Principle Study

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**Background:** Inhibition of soluble epoxide hydrolase (sEH) leads to an increase of endogenous epoxyeicosatrienoic acids resulting in the potentiation of their anti-inflammatory effects. High level of sEH has been associated with increased risk of anorexia nervosa, major depression disorder, hypertension, and pain. Thus, sEH has been hypothesized as a marker of diseased state in several health conditions. Traditional sEH quantification methods rely on expensive technologies such as radioactive system or mass spectrometry that are often unavailable by the bed-side. Recently, our group developed a novel ultrasensitive nanobody-based immunoassay (ELISA) for sEH protein quantification. This study aims to provide preliminary evidence of feasibility for this novel ELISA assay to be used in clinically relevant settings.

**Methods:** Five women with anorexia nervosa (3 White and 2 Asian. Mean age = 25.4) and five age- and BMI- matched healthy control women (3 White and 2 Asian, Mean age = 24.8) donated both fasting and two-hours-post-meal blood samples for this study. Using peripheral blood mononuclear cells (PBMC) isolated from blood samples, we measured sEH activity using a radioactive assay and quantified sEH protein using our nanobody-based immunoassay (ELISA). Sensitivity of the ELISA immunoassay was tested using systematic comparison of three different tracers in four ELISA formats. Specificity of the ELISA was evaluated against other epoxide hydrolases and detection of the target protein in human tissue homogenate samples. Using R 3.4.3 software, correlation between sEH activity and protein was tested using Spearman and Pearson methods, whereas analysis of the differences in sEH between anorexia nervosa and healthy control and between Whites and Asians was conducted using Wilcoxon rank-sum test and multivariate regression.

**Results:** Sensitivity analysis for the ELISA revealed an overwhelming advantage of PolyHRP as a label for nanobody based immunoassay. The use of PolyHRP as the tracer demonstrated a 141-fold increase in the sensitivity (0.21 OD•mL/ng) and 57-fold decrease in limit of detection (0.05 ng/mL) compared to classical ELISA. The correlation between sEH activity and protein was statistically significant in all samples combined ($r = 0.72$, p-value = 0.0001) and for subjects with anorexia nervosa ($r = 0.86$, p-value = 0.001). Moreover, the enhanced ELISA assay effectively detected the sEH protein over a larger linear range in PBMC, a biospecimen that sometimes yields insufficient sEH signal when testing using a radioactivity-based assay. Both sEH activity and protein were higher in anorexia nervosa compared to healthy controls, although not statistically significant (39.3 vs 16.4 cpm for activity; 77.11 vs 65.24 ng/mL for protein, AN vs. Ctrl). Subjects with anorexia nervosa displayed higher depression symptoms (total BDI = 21 vs 6.6, p-value = 0.0001), more anxiety (total BAI = 26 vs 3.6, p -value < 0.00001), and a higher decrease in post-meal anxiety (-2.8 vs -0.4, p-value = 0.019) in comparison to control women. Depression and anxiety showed trend of associations with sEH protein but not with sEH activity. Asian race was significantly associated with increased sEH activity (Mean: 52.94 vs 11.17 cpm, Asian vs White) and protein level (102.1 vs 50.56 ng/mL, Asian vs White) when analyzed with Wilcoxon rank-sum test (p-values = 0.0068 and 0.005) and multivariate regression (p-values = 0.05 and 0.024).

**Conclusions:** The novel ELISA demonstrated a significant correlation with the established radioactivity based sEH activity assay. Nanobodies permit high stability, ease of genetic manipulation, bacterial expression, and ability for continuous manufacture, therefore providing constant high-quality biochemical reagents. We have previously shown that sEH activity was increased in remitted anorexia nervosa compared to healthy control women. In this proof-of-principle study, we show that although not statistically significant, anorexia nervosa subjects displayed higher levels of both sEH activity and protein. The significant association of Asian race with sEH highlights the importance of considering the role heritable factors play in relationships between any "omics markers" and the disease of interest. Our ELISA assay has the capability to characterize large clinical samples with ease, therefore it can support the rigorous study designs needed to determine if sEH's inflammation modulatory function plays a key role in the pathogenesis of anorexia nervosa.

**Keywords:** Soluble Epoxide Hydrolase, Anorexia Nervosa, ELISA

**Disclosure:** Nothing to disclose.

### T100. A Molecularly Defined Insular --> Central Amygdala Circuit Controls Cue-Mediated Overconsumption

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**Background:** The ability to molecularly define cell types controlling complex behaviors would greatly enhance our ability to study these behaviors and their underlying circuitry. Feeding is a complex motivated behavior that is controlled not just by metabolic and homeostatic factors, but also by environmental factors such as emotion and the hedonic nature of the food itself. Yet, little is known about how brain regions involved in cognition and emotion might contribute to overeating, and therefore, obesity. In order to probe this neural circuitry, we recently developed and validated a simple and rapid Pavlovian context-induced feeding (Ctx-IF) task in which cues associated with food availability can later lead to increased food consumption in sated mice (Stern et al. Molecular Psychiatry 2018).

**Methods:** We used immediate early gene mapping to examine brain regions that are activated during Ctx-IF. We then used pharmacological and chemogenetic methods to inactivate the insular cortex (IC) and specifically the IC --> central amygdala (CeA) projection to determine whether this circuit was required for cue-mediated overconsumption. Lastly, we used the recently developed method, retro-TRAP (Retrograde - Translating Ribosome Affinity Purification), to profile projections from the IC to the CeA. We injected the retrograde canine adenovirus, CAV-GFP, into the CeA of SYN-NBL10 mice which contain anti-GFP–tagged ribosomal subunit proteins. Two weeks later, we dissected out the insular cortex and immunoprecipitated GFP, therefore pulling down polysome-bound, translating mRNAs of neurons that project to CeA. High-throughput RNA sequencing of samples allowed us to identify markers for this projection.

**Results:** In the Ctx-IF task, sated mice reliably consume more in the context previously paired with food than in the unpaired context. We found that the insular cortex and central amygdala,

among others, are activated in sated mice following the consumption test. Furthermore, we find that the insular cortex, and specifically, the IC -- > CeA projection, is required for overconsumption in the Ctx-IF task, but not for homeostatic feeding measured over 24 h. Using retro-TRAP, we then identified neuronal nitric oxide synthase 1 (nos1) and vesicular glutamate transporter 2 (slc17a6) as markers for this projection.

**Conclusions:** We have identified a molecularly defined circuit from the IC -- > CeA that controls cue-mediated overconsumption. Interestingly, the insular cortex is not involved in homeostatic feeding, i.e. food intake over a 24-hour period or food intake following an overnight fast. This indicates that there is top-down control of feeding that is independent of homeostatic regulation, which may be relevant to understanding the pathogenesis of obesity and/or binge-eating disorder.

**Keywords:** Obesity, Pavlovian Conditioning, Central Amygdala, Insular Cortex

**Disclosure:** Nothing to disclose.

### T101. Complex Functional Somatosensory and Basal Ganglia Network Properties in Anorexia Nervosa

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**Background:** Anorexia nervosa (AN) is a disease characterized by severe fear of weight gain that motivates dangerous weight loss behaviors. These features may result, in part, from disruptions in the ability to experience and/or respond to somato visceral sensations signaling motivational and emotional drives; exaggerated sensitivity to and over-correction to punishing stimuli; and disruptions in executive functions. As a result, AN has the highest mortality rate out of any psychiatric disease. Multimodal neuroimaging studies highlight atypical neural activity in brain networks associated with interoceptive awareness and processing of food properties (somatosensory network). Dysregulation of dopamine receptors within the basal ganglia, which plays a role in reward processing, may contribute to hyperactivity of aversive motivational systems and suppression of the appetitive systems as seen in AN. With recent advances in more efficient and computationally intense mathematical algorithms, it has become possible to apply graph theory to model the architecture of large-scale brain networks and characterize the network properties of individual brain regions. The most fundamental network measure is centrality or the connectedness of a particular region to other regions. Regions with high centrality are considered essential for information flow, and integrative processing. We hypothesized that children with AN compared to healthy controls (HC) would have differences in the centrality of key regions comprising the somatosensory and basal ganglia networks, and that these differences in centrality would be related to body dissatisfaction and drive for thinness in AN.

**Methods:** MRI resting state functional and gray matter scans were acquired in 22 children with AN and in 44 HC children. Data processing workflows were created using in house Matlab code. Regional parcellation was conducted using Freesurfer based on the Destrieux and Harvard Oxford atlases, and resulted in 74 bilateral cortical and 7 subcortical structures, including the cerebellum. CONN functional connectivity toolbox was used to process and construct the threshholded correlation matrix between the 165 cortical and subcortical regions. The Graph Theory GLM toolbox and in-house Matlab scripts were applied to subject-specific functional brain networks to compute local weighted network metrics indexing centrality (strength, betweenness centrality, and eigenvector centrality). To test for group differences, a contrast analysis within the generalized linear model covarying for age was performed. The regions investigated included somatosensory (thalamus, paracentral lobule and sulcus, primary somatosensory cortex, central sulcus, primary motor cortex, precuneus, secondary somatosensory cortex, supplementary motor area, middle and posterior insula) and basal ganglia (caudate, nucleus accumbens, pallidum, putamen, VTA/SN) networks. We controlled for multiple testing using a false discovery rate = 5% (significance: $q < 0.05$). Correlations between centrality and behavioral measures were conducted.

**Results:** 1. Subject Characteristics. There were 22 individuals with a history of AN at various phases of weight restoration (mean BMI = 19.83 kg/m2, sd = 2.48) and 44 HCs (mean BMI = 22.61 kg/m2, sd = 5.26). Compared to HCs, AN had greater body dissatisfaction scores ($F = 26.90$, $p = 9.00E\text{-}06$, $d = 1.75$) and greater drive for thinness scores ($F = 25.08$, $p = 1.60E\text{-}05$, $d = 1.69$). 2. Differences in centrality. Children with AN demonstrated lower centrality in various regions of the somatosensory network (paracentral lobule: $q = .04$, central sulcus: $q = .02$, posterior insula: $q = .02$, postcentral gyrus: $q = .02$, precentral gyrus: $q = .009$, SMA: $q = .002$). Individuals with AN demonstrated high centrality in the basal ganglia (caudate: $q = .03$, and in the nucleus accumbens: $q = .02$). 3. Associations of centrality measures with behavioral data: In AN, body dissatisfaction was negatively correlated to centrality of somatosensory regions (e.g. left postcentral gyrus: $r = -0.69$, $p = 0.03$, left precentral gyrus: $r = -0.86$, $p = 0.01$). Drive for thinness was also negatively correlated with centrality in various somatosensory regions (e.g. left thalamus: $r = -0.68$, $p = 0.04$), left precentral gyrus: $r = -0.55$, $p = 0.01$), and left postcentral gyrus: $r = -0.67$, $p = 0.006$).

**Conclusions:** The results demonstrate alterations in the functional network architecture of regions comprising the somatosensory and basal ganglia networks of children with AN. Findings indicate that AN is associated with increased communication between subcortical basal ganglia regions associated with dopamine signaling, and less information propagation was observed in somatosensory cortices. Such findings are consistent with the clinical presentation of AN, in which individuals seem "disconnected" from their bodies, using rigid rules (e.g., how much to eat or sleep) to guide behavior rather than bodily needs. This combined with a relentless drive to engage in behaviors that are valued (e.g., weight loss behavior) results in a driven pursuit of goals irrespective of emotional or physical consequence. Longitudinal studies will be required to address the question of causality between AN and network alterations and association with restricted ingestive behavioral patterns. These results suggest that network properties may serve as more sensitive central biomarkers and possibly predictors of outcome for AN treatments.

**Keywords:** Anorexia Nervosa, Graph Theory, Somatosensory Network, Basal Ganglia

**Disclosure:** Nothing to disclose.

### T102. Increased Sensitivity to Social Defeat Stress, Resulting in Depression-Like Behavior, in Mice With Conditional Forebrain-Specific Knockout of Ankyrin-G

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**Background:** ANK3 (which encodes Ankyrin-G protein) had been identified by Genome wide association studies (GWAS) as a major risk factor for bipolar disorder and schizophrenia. We previously generated a forebrain specific ankyrin-G knockout mouse model, which deletes all major forms of Ankyrin-G during adolescence. The homozygous knockout mice (Ank-G cKO) have behavioral features reminiscent of human mania, such as increased locomotor activity, decreased anxiety and decreased depression-like behavior. These behavioral changes were greatly ameliorated by anti-mania drugs, including Lithium and Valproic acid. After 14 days of social defeat stress, Ank-G cKO mice undergo a dramatic change to depression-like behavior, similar to socially defeated control. Unlike controls, which maintain the depression-like status, Ank-G cKO mice rapidly return to hyperactive status one week after stress. Repeated social defeat stress on Ank-cKO mice cause a dramatic switch between hyperactive and hypoactive status. We now sought to determine whether the heterozygous conditional knockouts (Ank-G Het KO) might show increased sensitivity compared to controls, with shorter periods of stress. In addition, we wanted to know whether Ank-G Het KO displayed sustained "depression-like" behavior, which can be used to test anti-depressant effects.

**Methods:** We characterized baseline behavior of Ank-G Het KO. We performed open field test for locomotor activity, elevated plus maze (EPM) and light dark box to test anxiety, forced swim test for depression-like behavior and Y maze test for cognition. Furthermore, we performed social defeat stress on Ank-G Het KO mice to induce its depression-like behavior. Following chronic stress, we tested effect of acute HNK (single dose, ip), chronic HNK (ip, once per day for three days) and chronic Fluoxetine (drinking water, 14 days) on chronically defeated mice. The effect of 4 days sub-threshold social defeat stress is also used to evaluate sensitivity to stress.

**Results:** Compared with controls which show dramatic difference from controls in many behavioral tests, there are very subtle differences between Ank-G Het KO and their littermate controls in almost all of the behavioral tests, including open field, elevated plus maze, light dark box, Y maze and forced swim test. Upon 14 days of chronic social defeat stress, as expected, both control and Ank Het cKO mice displayed depression-like behavior, including reduced locomotor activity in open field test, lower percent time in open arm of the in elevated plus maze test, and increased immobile time in forced swim test. In contrast to homozygous knockout, Ank-G het KO maintain "depression-like" behavior for a long time. Chronic Fluoxetine treatment rescued the depression-like behavior of both knockout and controls. However, single dose or triple doses of HNK did not have any effect on depression-like behavior of control or Ank-G Het cKO mice. Strikingly, Ank-G Het KO mice exposed to only 4 days of social defeat stress, mice displayed lower percent time in open arm of the elevated plus maze test, and increased immobile time in forced swim test, and thus were more sensitive to a short period of social defeat stress compared to controls.

**Conclusions:** These results indicate that Ank-G Het KO mice are more vulnerable to mild stress compared to controls. The depression-like behavior remains stable for weeks. These Ank-G Het cKO mice could be a valuable model to study mechanisms of depression-like behavior, and to test experimental therapeutics.

**Keywords:** ANK3, Depression-Like Behavior, Social Defeat Stress, Anti-Depressant

**Disclosure:** Nothing to disclose.

### T103. Mice Lacking Caspase 1, Interferon Gamma Receptor and Nitric Oxide Synthase 2 Genes Display Altered Depressive- and Anxiety-Like Behavior

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**Background:** Increasing evidence implicates neuroinflammatory pathways in the development and treatment response of MDD. Pre-clinical and clinical studies suggest that decreasing pro-inflammatory signaling may be beneficial to MDD. Dysregulation of three major inflammatory systems is evident in these conditions: A) increased oxidative stress by means of nitric oxide (NO) overproduction, driven by NOS2 (NO synthase 2), B) low-grade chronic pro-inflammatory status driven by caspase 1 (CASP1) overproduction, and C) interferon gamma (INFG) over production driven by type 1 T helper (Th1) cells.

**Methods:** The chronic unpredictable mild stress (CUMS) paradigm was used to evaluate whether male mice lacking the pro-inflammatory CASP1, INFG receptor, and NOS2 (Casp1, Ifngr, Nos2)-/- display altered depressive- and anxiety-like behavior. We also measured plasma adrenocorticotropic hormone (ACTH) and corticosterone (CORT) levels using enzyme-linked immunosorbent assay (ELISA).

**Results:** Triple knockout (Casp1, Ifngr, Nos2)-/- mice exhibit decreased depressive- and anxiety-like behavior and increased hedonic-like behavior and locomotor activity at baseline, and resistance to developing anhedonic-like behavior and a heightened emotional state following stress. Plasma ACTH and CORT levels did not differ between the triple knockout and wild-type mice following CUMS.

**Conclusions:** Our results show that simultaneous deficit in multiple pro-inflammatory pathways has antidepressant-like effects at baseline and confers resilience to stress-induced anhedonic-like behavior.

**Keywords:** Depressive-Like Behavior, Behavioral Despair, Anhedonia, Chronic Mild Stress, Corticosterone

**Disclosure:** Nothing to disclose.

### T104. Subcortical Shape Alterations in Major Depressive Disorder: Meta-Analytic Findings From the ENIGMA MDD Working Group

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**Background:** Major Depressive Disorder (MDD) has been posited to be characterized by alterations in subcortical structures that support cognitive and affective function, particularly in the hippocampus, amygdala, and striatum (Davidson et al., 2002). In a recent multi-site meta-analytic investigation by the ENIGMA-MDD consortium, researchers compared aggregate subcortical gray matter volumes between MDD patients and healthy controls. The main finding of this study was that MDD patients had smaller total hippocampal volume than did healthy controls, particularly patients with an adolescent age of onset ($\leq 21$ years) and recurrent episodes (Schmaal et al., 2016). Despite the large sample size and the use of harmonized analysis protocols, no other subcortical gray matter volumes distinguished patients with MDD from healthy controls. One possibility is that such aggregate measures of volume obscure or are insensitive to heterogeneous and complex localized effects. To this end, we conducted a multi-site meta-analysis on shape markers of subcortical brain structures in MDD, investigating group effects and correlations with key clinical characteristics.

**Methods:** Ten participating sites in the MDD Working Group of the ENIGMA consortium (Thompson et al., 2014; Schmaal et al., 2016; 2017) applied harmonized preprocessing and statistical

models on structural T1-weighted MRIs, yielding site-level summary statistics of subcortical volume and shape from 1779 MDD and 2962 healthy controls (CTL) individuals. Specifically, we used the ENIGMA Shape protocol to estimate radial distance – a metric of shape thickness – and the log of the Jacobian determinant – a metric of localized surface area reduction or enlargement (Gutman et al., 2012; 2015; Wang et al., 2011).

Each study site performed mass-univariate (per-vertex, per-measure) analyses for our primary and secondary statistical models. For our primary statistical models of interest, subcortical shape measures (thickness and surface area) were the outcome variables, and a binary group indicator variable (e.g., 0 = CTL, 1 = MDD) was the predictor of interest; age, sex (as a factor), and total ICV were covariates. Secondary analyses included comparisons between adolescent-onset (defined as ≤ 21 years; Schmaal et al., 2016; 2017) versus adult-onset MDD (and with CTL), and first- versus recurrent-episode MDD (both also compared to CTLs). In an exploratory analysis, we conducted continuous regression analyses with depressive symptom severity at the time of scan.

Resulting group-level maps of effect sizes (i.e., Cohen's d for group comparisons and Pearson's r for continuous regression) were aggregated for mass-univariate meta-analysis. Specifically, for each vertex point, we pooled each site's effect sizes, utilizing an inverse variance-weighted random-effects model fit with restricted maximum likelihood estimation using the R package metafor (van Erp et al., 2018; Schmaal et al., 2016; 2017). This resulted in meta-analytic maps of p-values; we corrected for multiple comparisons by using a modified searchlight false discovery rate (FDR) procedure set to $p < 0.05$ (Kriegeskorte et al., 2006; Langers et al., 2007). For each linear model, FDR correction globally across all 7 bilateral subcortical regions (by setting the Euclidean distance between vertices of different subcortical regions to infinity) and two measures (thickness, surface area), as well as separately for each bilateral region.

**Results:** In global FDR-corrected analyses, relative to CTL, patients with adolescent-onset MDD had reduced thickness and surface area primarily in the subiculum of the hippocampus and the basolateral amygdala (all d ≤ -0.164). Relative to first-episode MDD, recurrent MDD patients had reduced thickness and surface area in the CA3/dentate gyrus of the hippocampus and lateral amygdala (all d ≤ -0.173) and increased thalamic thickness and surface area (all d ≥ 0.176). In local FDR-corrected analyses, relative to CTL, MDD had reduced thickness in the caudate head and reduced surface area of the subiculum of the hippocampus, lateral amygdala, and nucleus accumbens (all d ≤ -0.108). Relative to CTL, recurrent MDD patients had reduced amygdala thickness and surface area, and reduced nucleus accumbens surface area (all d ≤ -0.125), whereas first-episode MDD had reduced hippocampal and caudate surface area (all d ≤ -0.154). We found no significant interactions between diagnosis and age or sex; further, subcortical shape measures were not associated with severity of current depressive symptoms.

**Conclusions:** We identified robust reductions in the thickness and surface area of the subiculum of the hippocampus in patients with an earlier onset of illness. The subiculum is a stress-sensitive subregion of the hippocampus that contains a higher density of glucocorticoid binding sites relative to other hippocampal subfields (Lucassen et al., 2001; Tao et al., 2011). We also observed reductions in the thickness and surface area of the basolateral amygdala, which is primarily involved in evaluating emotional content of sensory inputs (Mosher et al., 2010; Rubinow et al., 2016); these effects appear to be driven by recurrence of illness. In conclusion, applying a novel shape analysis pipeline in the largest investigation to date of subcortical shape in MDD enabled us to detect finer-grained alterations in thickness and surface area of subcortical structures that may represent important endophenotypes of MDD.

**Keywords:** Subcortical Shape Analysis, Major Depressive Disorder (MDD), ENIGMA Working Group, Hippocampus, Amygdala

**Disclosure:** Nothing to disclose.

## T105. Examining the Role of Microbiota in Emotional Behavior: Antibiotic Treatment Exacerbates Anxiety in High Anxiety-Prone Rats

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**Background:** Gastrointestinal disorders are highly comorbid with affective disorders such as Major Depression Disorder. An emerging view of gut microbiota highlights their roles in normal development and maintenance of a healthy gut as well as its broader effects on the immune system, central nervous system function and organismal health overall. Recent rodent studies indicate that manipulating microbiota influences brain function and emotional behavior, effects that may be mediated via changes in integrity of the blood-brain barrier. The present study builds on this work using a model of individual differences in temperament where rats were selectively bred for high versus low novelty seeking behavior. Low Novelty Responder (LR) rats exhibit high levels of anxiety- and depression-like behaviors compared to High Novelty Responders (HR). HR/LR rats display a range of neurochemical and behavioral differences. In the current study, we hypothesized that some aspects of the HR/LR neurobehavioral phenotypes may be driven, at least in part, by differences in their microbiomes and that altering the microbiota profile of the strains could shift aspects of their behavior.

**Methods:** To test the hypothesis that LR rats' high levels of anxiety- and depression-like behavior are linked to altered microbiome composition (compared to low anxiety HR rats), we first treated adult male HR/LR rats with an antibiotic cocktail aimed at depleting the microbiome to test if changes in microbiota altered HR/LR emotional behavior. Then, 16 s RNA was extracted from fecal samples and we applied next generation sequencing to profile the microbiome in adult male HR/LR animals at baseline and following antibiotic treatment. Given the known connections between the microbiome and immune system, we also analyzed levels of several cytokines/chemokines in HR/LR rats at baseline and following antibiotic treatment to determine any potential correlations between immune markers, microbiome components, and/or behavioral measures.

**Results:** We found that antibiotic treatment exacerbated HR/LR behavioral differences, increasing LRs' already high levels of anxiety-like behavior while reducing the already low levels of passive coping in HRs. We found significant correlations between distinct behavioral domains and levels of several gut microbial species as well as levels of circulating cytokines.

**Conclusions:** The overarching goal of these studies was to help elucidate the relationship between the microbiome, immune response, and an individual's emotional phenotype to facilitate development of novel hypotheses regarding the biology of depression and anxiety. While we did not find many differences in microbiome in HR/LR rats, we did find novel correlations between microbiota species, peripheral immune factors, and distinct measures of rodent emotional behavior, which may ultimately shed light on biological mechanisms relevant to human psychiatric condition.

**Keywords:** Gut Microbiome, Anxiety, Depression, Cytokine

**Disclosure:** Nothing to disclose.

## T106. Regionally-Selective Knockdown of Astroglial Glutamate Transporters in Infralimbic Cortex Increases Local Excitatory Neurotransmission and Evokes a Depressive Phenotype in Mice

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**Background:** Increases in energy metabolism together with disturbances of astrocyte number/function in the ventral anterior cingulate cortex (vACC) have been suggested as important contributors to the pathophysiology of major depressive disorder (MDD). Hence, the functional hyperactivity reported in vACC may result from a reduced glutamate clearance from excitatory synapses and a subsequent increase in neuronal excitation. Astrocytes are emerging as essential players in synaptic function, controlling extracellular levels of ions and neurotransmitters, responding to them, and releasing gliotransmittters which regulate synaptic transmission and plasticity. In particular, the astroglial glutamate transporters GLT-1 and GLAST are responsible for the reuptake of more than 90% from central glutamatergic excitatory synapses, thereby directly controlling neuronal excitability. Here we examined the functional and behavioral consequences of knocking down GLT-1 and GLAST with RNAi strategies in mouse infralimbic cortex (IL) under the working hypothesis that a functional hyperactivity in IL may result in a depressive-like phenotype.

**Methods:** Pentobarbital-anesthetized mice (C57BL/6 J) were unilaterally microinfused in IL with siRNA (small interfering RNA) targeting GLAST or GLT-1 at a single dose of 60 µg/µl; 4.2 nmol/dose). Mice were sacrificed at 1, 3 or 7 days after siRNA infusion. Electrophysiological recordings from layer V pyramidal neurons were made in cortical slices containing the IL using whole-cell configuration of the patch-clamp technique. Behavioural assessments included the forced swim test (FST), tail suspension test (TST) and the sucrose preference test (SPT, anhedonia). Immunohistochemistry and in situ hybridization were used to assess changes in the expression of GLAST and GLT-1, and of other mRNAs/proteins (see below). Changes in 5-HT release were determined by intracerebral microdialysis in freely-moving mice. Changes induced by GLAST/GLT-1 knockdown on most variables were assessed 24 h after siRNA microinfusion. Statistical analyses were performed with $N = 8$-10 animals/group and by two-tailed Student's t-test and one-way or two-way ANOVA followed by Tukey's post-hoc test as appropriate Statistical significance has been set at the 95% confidence level.

**Results:** Unilateral microinfusion of a pool of siRNA sequences targeting GLAST or GLT-1 in mouse IL induced a moderate (20-30%) and long-lasting (7 days) decrease in their mRNA and protein expression ($p < 0.0001$). Intra-IL GLAST/GLT-1 siRNA infusion also reduced glial fibrilary acidic protein (GFAP)-positive astrocyte density and increased excitatory neurotransmission in layer V pyramidal neurons, as shown by an increased resting membrane potential ($p < 0.005$), increased evoked discharge rate ($p < 0.0001$), slow down of evoked EPSC ($p < 0.003$), as well as increased spontaneous EPSC (sEPSC) amplitude and frequency ($p < 0.0001$). GLAST/GLT-1 knockdown also increased gliotransmission, as shown by the higher amplitude and frequency of slow inward currents (SICs) ($p < 0.0015$), mediated by extra-synaptic NMDA-R. Moreover, GLAST/GLT-1 knockdown evoked a depressive-like phenotype, as assessed by the FST ($p = 0.0003$), TST ($p < 0.0001$) and SPT ($p < 0.0005$), which was reversed by the acute i.p. administration of citalopram and ketamine 30 min prior to the test. GLAST or GLT-1 knockdown in IL markedly reduced serotonin (5-HT) release in the dorsal raphe nucleus (DR) and induced an overall reduction of brain derived neurotrophic factor (BDNF) expression in cortical and hippocampal areas of both ipsilateral and contralateral hemispheres. BDNF reductions were highly correlated with the reduction of GLAST and GLT-1 mRNA expression in IL ($p < 0.0001$). Moreover, Egr-1 (early growth response protein-1) labelling suggests that both siRNAs enhance the putative GABAergic tone onto DR 5-HT neurons, leading to an overall decrease of 5-HT function, likely related to the widespread reduction on BDNF expression. Remarkably, similar reductions of GLAST and GLT-1 expression in the neighboring prelimbic cortex (PrL) did not induce a depressive-like phenotype in any of the tests examined and did not alter 5-HT release in DR.

**Conclusions:** These results show that a moderate reduction of glutamate clearance by astrocytes in IL results in very marked local and distal changes in neuronal activity, likely associated to the depressive-like phenotype evoked by GLAST/GLT-1 knockdown in IL. The increased excitatory neurotransmission in layer V pyramidal neurons of IL, together with the reduced serotonergic function suggests that the depressive-like phenotype induced by GLAST/GLT-1 knockdown results from an excessive top-down inhibitory control of DR neurons by the IL. Hence, a focal glial change in IL translates into global change of brain activity by virtue of the descending projections from IL to DR and the subsequent attenuation of serotonergic activity.

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**Keywords:** Astrocytes, Major Depression Disorder, Glutamate, Infralimbic Cortex, Serotonin

**Disclosure:** Nothing to disclose.

## T107. Developmental Omega-3 Fatty Acid Deficiency Impairs Fear Extinction in Adult Rats: Prevention by Early Omega-3 Polyunsaturated Fatty Acid Supplementation

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**Background:** While traumatic life events can precipitate anxiety (PTSD) and mood disorders, associated risk and resilience mechanisms remain poorly understood. Emerging translational evidence suggests that patients with PTSD and mood disorders exhibit deficits in omega-3 polyunsaturated fatty acids, including docosahexaenoic acid (DHA, 22:6n-3), and that development deficits in brain DHA accrual can lead to enduring structural and functional abnormalities in rat and non-human primate brain. Reciprocal connections between the infralimbic prefrontal cortex (PFC) and the basolateral amygdala mediate conditioned fear acquisition and extinction, and this circuit undergoes robust maturational changes during postnatal development. The present study evaluated the hypothesis that deficits in brain DHA accrual during perinatal development would lead to perturbations in fear expression and extinction in young adulthood and investigated whether postnatal supplementation with omega-3 polyunsaturated fatty acids (fish oil) could prevent this.

**Methods:** Female rats were fed either a control diet (CON) containing the omega-3 fatty acid alpha linoleic acid (ALA, 18:3n-3), or a diet containing no omega-3 fatty acids (deficient, DEF) 30 days prior to mating through gestation and lactation. On postnatal day 21, male pups from CON litters were weaned onto CON diet ($n = 8$), and pups from DEF litters were either maintained on DEF diet ($n = 11$) or switched to a diet supplemented with 1.1% fish oil (FO)($n = 11$) to adulthood (P90). In adulthood novelty-induced locomotor activity was evaluated in automated activity

chambers, followed by a three-day cued fear conditioning protocol. On day 1 (fear acquisition), rats received habituation tones followed by seven tones co-terminating with a foot shock and freezing behavior recorded. On day 2, contextual conditioning was evaluated as freezing in the chamber prior to the first tone, and then rats received 20 unpaired tones to assess fear extinction (day 2) and extinction recall (day 3). Kaplan-Meier survival analyses were used to compare group differences in acquisition and extinction rates. Postmortem PFC DHA composition was determined by gas chromatography.

**Results:** Adult rats maintained on the DEF diet during perinatal development exhibited significantly lower PFC DHA levels compared with rats maintained on the CON diet, and adult DEF rats switched to fish oil-supplemented diet on P21 exhibited PFC DHA levels that were similar to CON rats. There were no group differences in novelty-induced locomotor activity. During fear acquisition training, there was a non-significant trend for faster acquisition by DEF and FO rats compared with CON rats ($p = 0.059$). While DEF and FO rats exhibited numerically greater freezing to context, this did not reach statistical significance. During fear extinction, DEF rats took significantly longer to achieve extinction relative to both CON and FO rats ($p = 0.019$), and CON and FO rats were not statistically different. There were no group differences during extinction recall.

**Conclusions:** Uncorrected deficits in brain DHA accrual during perinatal development cause a selective impairment of fear extinction, and prior omega-3 polyunsaturated fatty acid supplementation prevents this impairment. These findings suggest that developmental omega-3 fatty acid deficiency disrupts the maturation of neural circuits that mediate fear extinction and may represent a modifiable risk factor for fear extinction deficits associated with anxiety and mood disorders.

**Keywords:** Omega-3 Fatty Acids, Development, Fear Conditioning

**Disclosure:** Nothing to disclose.

### T108. The Role of Microglia in Synaptic Rewiring by Early-Life Adversity

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**Background:** Early-life adversity can have a profound and lifelong impact on an individual's risk for emotional disorders such as depression by modulating the maturation of brain circuits. We find that early-life exposure to an impoverished environment and unpredictable maternal care (in a limited bedding and nesting [LBN] paradigm) provokes major alterations in cognitive and emotional responses to subsequent stress, as well as anhedonia, accompanied by aberrant connectivity between the hippocampal-limbic system and reward/pleasure-related regions. Within the hypothalamus, early-life adversity in the LBN model causes an increase in the number of excitatory synapses onto corticotropin-releasing hormone (CRH)-expressing neurons in the paraventricular nucleus (PVN). Further, such synaptic changes suffice to induce large-scale and enduring epigenomic changes in the expression of neuronal genes, including Crh. However, the mechanisms by which early-life adversity modulates synapse development or persistence in developing brain circuits remain unknown. We hypothesize that microglia contribute to normal synaptic reduction on CRH neurons in the developing PVN, and that adverse early-life experiences interfere with this function.

**Methods:** To interrogate microglial function, we employed dual-reporter transgenic mice with visible CRH neurons and microglia and two-photon time-lapse imaging in acute slices of the PVN. We obtained these hypothalamic slices from control and LBN male mice at postnatal day (P)8 and visualized live microglial process dynamics and interactions with CRH neurons. To probe the molecular mechanisms of microglial-neuronal interactions in the developing PVN, we bath-applied CRH to P8 slices and analyzed the impact on microglial process dynamics. In fixed tissue, we utilized 3D-reconstruction confocal microscopy and immuno-detection of synaptic markers to visualize in high-resolution the developmental trajectory of synaptic engulfment by microglia in the PVN.

**Results:** Early-life adversity augmented the number of vGlut2 + /PSD95 + excitatory synapses onto CRH neurons by the end of the LBN experience (P10), without altering the number of CRH neurons or microglia in the PVN at P4, P8, or P10. However, microglial processes overlapped more substantially with CRH neurons at P8 than P4, potentially indicating a period of greater microglial-neuronal interaction. Live-imaging revealed that microglial process dynamics were diminished at P8 in the PVN of LBN mice, and bath application of CRH recapitulated this effect of early-life adversity on microglial function. Characterization of the effects of early-life adversity on microglial synaptic engulfment is currently ongoing, along with the exploration of potential sex differences.

**Conclusions:** Microglia are potential contributors to early-life experience-dependent synaptic rewiring of stress-sensitive neurons, and future manipulation of microglial function during development may prevent stress-related emotional disorders in adulthood, thereby providing novel targets for therapeutics or preventative interventions.

**Keywords:** Early Life Stress, Early Life Adversity, Microglia, CRH, Hypothalamus

**Disclosure:** Nothing to disclose.

### T109. Lithium Reverses Presynaptic GABAergic Signaling Deficits in the ANK3 W1989R Mouse Model

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**Background:** Multiple genome-wide association studies (GWAS) have shown that the ANK3 gene is one of the most significant risk loci for bipolar disorder (BD). The ANK3 gene encodes ankyrin-G, an adaptor protein that is involved in the formation of the axon initial segment (AIS), nodes of Ranvier, and GABAergic synapses. Recently, we have generated a mouse model with a W1989R mutation in Ank3, which abolishes the interaction between ankyrin-G and GABARAP necessary for ankyrin-G-¬dependent stabilization of postsynaptic GABAA receptors. We have shown that the Ank3 W1989R mice have striking reductions in inhibitory currents in cortex and hippocampus compared to control mice resulting in increases in the intrinsic excitability of pyramidal neurons. Importantly alterations in inhibitory signaling have also been seen in BD patients. Consistent with this idea, we recently identified a BD family carrying the ANK3 W1989R variant in our patient cohort in the Heinz C. Prechter Bipolar Research Program at the University of Michigan. The proband is a Caucasian male with type I BD characterized by recurrent mania and depression with a successful treatment with lithium.

**Methods:** In these studies, we have treated Ank3 W1989R mice for 21 days with chow containing lithium carbonate until serum levels reach the therapeutic range and used voltage clamp and

current clamp whole cell electrophysiology recordings to measure inhibitory postsynaptic currents in cortical and hippocampal pyramidal neurons.

**Results:** Our results showed a 21-day lithium treatment partially reverses the defect in spontaneous inhibitory post-synaptic current (sIPSC) frequency, while not significantly affecting sIPSC amplitude. Since sIPSC frequency is a measure of presynaptic GABA release probability, we hypothesize that lithium is increasing activity of parvalbumin-positive GABAergic interneurons.

**Conclusions:** In summary, these results suggest that the ANK3 has an important role in the control of cortical and hippocampal neuronal excitability and dysfunction of this pathway may contribute to the imbalance of circuits seen in BD patients. In addition, our work suggests that lithium may act to increase the presynaptic GABA release in our model, perhaps resulting from increased excitability of parvalbumin-positive interneurons.

**Keywords:** ANK3, Bipolar Disorder, GABA, E/I imbalance, Lithium Response

**Disclosure:** Nothing to disclose.

### T110. Co-Expression Network Modeling Identifies Key Long Non-Coding RNA and mRNA Modules in Altering Molecular Phenotype to Develop Stress-Induced Depression in Rats

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**Background:** Long non-coding RNAs (lncRNAs) has recently emerged as one of the critical epigenetic controllers, which participate in several biological functions by regulating gene transcription, mRNA splicing, protein interaction, etc. In the present study, we examined how lncRNAs are co-expressed with gene transcripts and whether specific lncRNA/mRNA modules are associated with stress vulnerability or resiliency to develop depression.

**Methods:** Differential regulation of lncRNAs and coding RNAs were determined in hippocampi of 3 group of rats (male rats; 6 rats per group) comprising learned helpless (LH, depression vulnerable), non-learned helpless (NLH, depression resilient) and tested controls (TC) using a single microarray-based platform. Weighted gene co-expression network analysis (WGCNA) was conducted to correlate the expression status of protein-coding transcripts with lncRNAs. The associated co-expression modules, hub genes, and biological functions were analyzed. To further explore the relationship between the depression-related hub lncRNAs and hub mRNAs, Canonical correlation analysis (CCA) analysis was performed.

**Results:** lncRNA datasets were used to compare LH vs TC, NLH vs TC, and LH vs NLH groups. Among the 3 comparisons, the most notable changes were noted in the LH group relative to TC group, as we found 729 differentially regulated lncRNAs including 346 up and 383 downregulated. With these 729 lncRNAs, WGCNA provided 4 modules: LTCblue LTCbrown, LTCturquoise, and LTCyellow. However, only LTCblue module showed negative association with the LH phenotype. LH vs NLH comparison showed 314 DEGs. Among them, 62 were upregulated and 252 were downregulated. Three modules were observed based on the topology network analysis: LNCbrown, LNCblue, and LNCturquoise, which demonstrated a statistically significant association with NLH phenotype. Brown module was positively associated with changes in phenotypic trait, whereas blue and turquoise modules were negatively correlated. NLH vs TC group comparison showed five individual modules (NTCgreen, NTCturquoise, NTCyellow, NTCred, NTCblue, NTCbrown). NSCturquoise was identified as the most significantly associated module with resiliency (NLH). Interestingly, pathway analysis demonstrated that several modules from LH vs TC comparison (blue, brown, turquoise, and green) and LH vs NLH comparison (blue, brown, turquoise; and blue and turquoise) were significantly associated with the olfactory transduction. Also, GO analysis suggested that these modules were significantly associated with sensory perception, detection of chemical stimulus, and response to stimulus.

**Conclusions:** Altogether, we found signature co-expression networks that underlie the normal as well as aberrant response to stress. We constructed modules and analyzed the association between the modules with different phenotypes. These modules were enriched in the olfactory transduction. We also identified hub and specific driver genes associated with vulnerability and resilience. Altogether, our study provides solid evidence that these complex trait specific networks may play a crucial role in resiliency or vulnerability to develop depression. Deciphering lncRNA functions and linking their role to underlying pathophysiological mechanisms is needed to further identify the specific involvement of lncRNAs in depression pathogenesis.

**Keywords:** Long Non-Coding RNAs, Hippocampus, Rat, Depression, Vulnerability

**Disclosure:** Nothing to disclose.

### T111. Testosterone Drives Circuit-Specific Sexual Dimorphism in Responses to Stress

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**Background:** Depressive disorders disproportionately affect women, and the symptoms of these disorders are sexually dimorphic, with female patients more likely to experience anhedonia and social withdrawal. The ventral hippocampus (vHipp) regulates emotional and motivated behaviors through projections to nucleus accumbens (NAc) and amygdala (BLA), and the vHipp-NAc circuit is critical for resilience to stress in male mice (Bagot et al., 2015). However, sex differences in this circuit and the potential role of this circuit in female susceptibility to the stress resilience and depression are unknown. Using a subchronic variable stress (SCVS) paradigm that causes depression-like effects in female but not male mice (Hodes et al., 2015), we investigate stress-induced alterations in the function of vHipp-NAc projection cells underlying depression-like behavior in male and female mice and provide evidence that neuronal excitability in this circuit drives sex differences in behavior.

**Methods:** Male and female C57Bl6/J mice from Jackson Labs were used in this study and all experiments were performed in accordance with Michigan State University IACUC-approved protocols. SCVS was performed essentially as previously described (Hodes et al., 2015; Brancato et al., 2017). Briefly, mice underwent variable stress each day for six days, consisting of one hour of foot shock, tail suspension, or restraint, twice each in alternating order. For assessment of depression- or anxiety-like behaviors, the open field apparatus consisted of a custom-made, square white polyvinylchloride foam box (38 cm × 38 cm × 35 cm) and elevated plus maze was performed using a custom-built apparatus based on plans from ANY-maze (www.anymaze.com). Viral vectors were injected by stereotaxic surgery into vHipp (3° angle; -3.2 mm anteroposterior (AP), ± 3.4 mm mediolateral (ML) and -4.8 mm dorsoventral (DV)), NAc (10°; + 1.6 AP; ± 1.5 ML; -4.4 DV), or BLA (0°; -1.3 AP; ± 3.4 ML; -4.5 DV). For electrophysiology, coronal slices containing vHipp (250 µm thick) were cut in ice-cold sucrose artificial cerebrospinal fluid (ACSF), and recordings were performed at 30–32 °C in ACSF. vHipp was identified under visual

guidance using infrared differential interference contrast video microscopy with a 40 × water-immersion objective (Olympus BX51-WI). Whole-cell voltage-clamp recordings were performed with a computer-controlled amplifier (MultiClamp 700B), digitized (Digidata 1440), and acquired with Axoscope 10.1 (Molecular Devices) at a sampling rate of 10 kHz.

**Results:** We demonstrate that female mice have increased excitability of vHipp-NAc neurons compared to males at baseline. We show that adult loss of testosterone in male mice conveys a similar difference in function of this circuit, and that application of testosterone in female mice can reduce the excitability of the circuit to male levels. In addition, we show that the behavioral differences in response to SCVS in males and females are also dependent upon adult testosterone. Finally, we investigate the causal role of the vHipp-NAc projections neurons in the sexually dimorphic response to SCVS using circuit-specific DREADD manipulation of vHipp-NAc excitability.

**Conclusions:** We demonstrate higher baseline excitability of vHipp-NAc projection neurons in females compared to males and present evidence that this may underlie increased vulnerability to stress. Moreover, we show that testosterone drives these differences, suggesting that manipulation of androgen receptor function or downstream signaling or gene expression changes may present a potential target for sex-specific treatment of depression or stress-related disorders.

**Keywords:** Neurocircuits, Mood Disorders, Sex Difference

**Disclosure:** Nothing to disclose.

### T112. Biological Changes in a Pharmacologically–Induced Depression Model Confirm the Role of Estrogen Sensitivity in Perinatal Depression

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**Background:** Hormonal transition phases trigger severe depressive episodes in some women. We do not know why. Intriguingly, recent preclinical human model work of ours provide direct evidence for sex-hormone manipulation to provoke subclinical depressive symptoms in about 12% of healthy volunteers, a phenomenon that was coupled to increases in serotonin transporter binding (which lowers synaptic serotonin) and was dependent on the biphasic estradiol fluctuation [Frokajer et al 2015, Bio Psy]. Further, earlier work of ours, where we analyzed dynamic gene transcription profiles in a clinical cohort of women across natural pregnancy and pre- to postpartum transition, suggest that enhanced sensitivity to oestrogen signaling may drive increased risk for depressive symptoms [Mehta et al 2014, Psych Med].

Here, we test if this earlier identified set of 116 sex-steroid responsive transcripts, which predicted perinatal depression (PND) translates to our pharmacological model of hormone-induced mood changes. If so, this strongly would strengthen the estradiol sensitivity hypothesis and highlight estradiol sensitivity as a promising biomarker for stratification and personalized medicine approach to hormone-induced depressive episodes.

**Methods:** Longitudinal genome-wide gene expression and DNA methylation data from 60 women exposed to a Gonadotrophin Releasing Hormone agonist (GnRHa) or placebo [1] were generated. Treatment with GnRHa induces a biphasic hormonal transition phase reflected by an initial stimulation of the hypothalamic–pituitary–gonadal axis, a desensitization of the pituitary GnRH receptors, and subsequently a suppression of the ovarian sex-steroid production to menopausal level, which is reached within 10-14 days and sustained for 28 days' post intervention. This use of GnRHa mimics core features of the perimenopausal transition, i.e., excessive fluctuations in ovarian hormones particularly estradiol. Moreover, the GnRHa model also partly reflects the physiological changes from pre to post-partum, which is characterized by a rapid decline from high levels of hormones such as estrogen established during pregnancy. Differences between baseline and follow-up points for gene expression and DNA methylation in the biphasic ovarian response to GnRHa were assessed using linear mixed effects models. The study was registered at www.clinicaltrials.gov (ID: NCT02661789).

**Results:** Of our 116 a priori defined PND predictive transcripts [2], a significant (19%) overlap was observed with those differentially expressed post-GnRHa at both early and later follow-up indicating sustained effects. Similarly, 49% of tested genes were differentially methylated post-GnRHa at the late follow-up point. Within the GnRHa group, a large proportion of PND genes were significantly associated (gene expression; DNA methylation) with changes in depressive symptoms (28%; 66%), estradiol levels (49%; 66%) and neocortex serotonin transporter binding (8%; 45%) between baseline and later follow-up.

**Conclusions:** Our data bridge clinical PND biomarkers with a pharmacological model of sex hormone-induced mood changes and directly relate estrogen-induced biological changes with depressive symptoms and associated serotonin signaling changes. Our data highlight that individual variations in molecular sensitivity to estrogen associate with susceptibility to hormone-induced mood changes and hold promise for candidate biomarkers, which could help inform personalized prevention in high-risk groups or treatment of manifest depressive episodes related to hormonal transitions.

**Keywords:** Depression Subtypes, Sex Steroids, Gene Expression

**Disclosure:** Nothing to disclose.

### T113. Self-Reported Burden of Illness in Patients With Recurrent Major Depressive Disorder Who Recently Responded to an Oral Antidepressant: Relationship to Clinician Assessments of Relapse

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**Background:** Burden of Illness (BOI) associated with Major Depressive Disorder (MDD) includes presence of neurovegetative symptoms and cognitive distortions, symptom severity, functional impairment, reduced quality of life, and frequent comorbid conditions including pain. Increased burden of illness may predict risk for poor outcomes. The BOI measure was initially proposed by Ishak W.W., et al. [1] in 2013 and used principal component analysis (PCA) to combine self-report measures involving symptom severity, functioning and quality of life. In our proposed work, we extended the BOI measure to include other aspects of BOI, namely, stress, sleep quality, pain, anxiety and anhedonia. The present analyses consider the relationship between "baseline" patient-reported BOI and subsequent relapse defined using the clinician-rated Montgomery-Asberg Depression Rating Scale (MADRS) during the ongoing OBSERVEMDD0001 (NCT02489305) observational trial of patients with recurrent MDD, who had recently responded to oral antidepressant treatment.

**Methods:** Patients (N = 330) with recurrent MDD who recently (within 3 months of trial enrollment) responded to oral antidepressant treatment for a Major Depressive Episode, were

clinically stable with MADRS ≤ 14 at study screening, and receiving ongoing, clinician-directed treatment with oral antidepressant were enrolled. Of these, 286 patients (MADRS relapse = 74, non-relapse = 212) with data from self-report measures at baseline were included. A Kaiser-Meyer-Olkin test was performed to assess the level of shared variance among the total score from self-report measures namely, Quick Inventory of Depressive Symptomatology-Self Report 16-Item Scale (QIDS-SR16); World Health Organization Disability Assessment Schedule 2.0 (WHODAS 2.0) 12 item version; Pain, Frequency, Intensity and Burden Scale (P-FIBS); Perceived Stress Scale (PSS); Snaith Hamilton Pleasure Scale (SHAPS); 7-item Generalized Anxiety Disorder Scale (GAD-7); Medical Outcomes Study Sleep Scale-Revised (MOS Sleep-R); and Recent Life Changes Stress Test (RLCST) together with the EuroQol Group 5-Dimension 5-Level (EQ-5D-5L) utility index score. All the self-report measures scored ≥ 0.84 on a scale of 0-1 indicating a strong shared variance among the self-report measures. We adopted the method described in Ishak W.W., et al. 2013 [1] to define BOI. To this end each self-report total score was normalized using z-transformation. A principal component analysis (PCA) was performed and the factor loadings corresponding to the 1st principal component (PC1) (accounting for 0.52 percent of the total variance) were used as weights in combing the self-report measures. The BOI measure was calculated as the summation of all normalized self-report measures multiplied by its corresponding factor loadings. The factor loadings for GAD-7, P-FIBS, PSS, QIDS-SR16, RLCST, SHAPS, WHODAS 2.0, MOS Sleep-R, EQ-5D-5L were 0.81, 0.64, 0.79, 0.83, 0.47, 0.58, 0.85, -0.62, and 0.81, respectively. The BOI was then divided by its total variance to normalize its range. The t-statistic was used to compare the baseline BOI of patients who relapsed as compared with that of the patients without relapse.

**Results:** The mean baseline BOI score demonstrated higher burden (0.2336) for patients who relapsed and was significantly different from those who did not relapse (−0.0815) and had lower burden, with t-statistic (equal variance) = −2.3531 and p-value = 0.0193.

**Conclusions:** The current findings indicate that patients with MDD reporting increased BOI at baseline are at greater risk for subsequent relapse based on a clinician-rated measure. The factor loadings (or weights) obtained in this cohort need to be validated in an independent cohort to assess the generalizability of these results.

**Keywords:** Major Depressive Disorder, Burden of Illness, Relapse

**Disclosure:** Johnson & Johnson, Employee

**References:**

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## T114. Clinical Characteristics and Biomarker Correlates of Global Functioning in Offspring of Bipolar Parents: A Preliminary Analysis

*Maura Furey, Giacomo Salvadore, Ziad Saad, Derrek Hibar, Lynn Yieh, Julia Lull, Stephanie Rassnick, W. Kyle Simmons*, Hartmuth Kolb, Gahan Pandina*

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**Background:** Evidence suggests that it is important to identify individuals at risk for developing bipolar disorder (BD) before illness is fully manifest. Disability associated with BD increases at age 15 to 19 years, becoming increasingly severe up to 25 to 29 years, suggesting that psychosocial impairment may be used to identify patients who are likely to show a deteriorating course of illness. This functional decline may manifest prior to BD and, along with other clinical signs and biomarker changes, precede a first manic episode. The Aretaeus Study, initiated by Janssen Research and Development, uses a longitudinal observational design to characterize psychosocial and biological predictors of BD onset and prognosis in those at a high familial risk for the illness. The goal of the current analysis was to validate the utility of the Global Assessment of Functioning (GAF) as a risk variable in this dataset and identify a biomarker signature that predicts psychosocial functioning as well as specific prodromal BD clinical features such as mania and depression.

**Methods:** Subjects age 15- to 25-years who are offspring of parents with BD were assessed every 3 months (either in the clinic or via telephone) for up to 24 months. Participants were either drug-naïve or on stable treatment for more than 4 weeks prior to entering the study and did not have BD or psychosis at the time of study intake. GAF was assessed at all study timepoints by a trained clinician using the modified GAF scale (Hall, 1995, Psychosomatics). GAF was scored on a numeric scale from 1 to 100, with higher scores indicating better functioning. Participants were assigned to one of two groups based on their psychosocial functioning at screening: those with at least mild impairment in psychosocial functioning (GAF ≤ 70) vs. those with no impairment in psychosocial functioning (GAF > 70). A variety of clinical assessments were utilized to assess diagnosis and subthreshold symptoms of mania and depression, including the Longitudinal Interval Follow-Up Evaluation (LIFE) which yields a Psychiatric Status Rating (PSR) for various DSM-IV psychiatric disorders, and the General Behavioral Inventory. Patient-reported outcomes of depression, mania, sleep, anxiety, and other domains were also collected, primarily via smart-phone.

Blood plasma collected at baseline was used to assess Adrenocorticotropic hormone (ACTH) and kynurenine pathway metabolites. Serum was used for assessment of C-reactive protein (CRP), cortisol, interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-alpha), mature brain derived neurotrophic factor (BDNF), leptin, and adiponectin. Finally, nighttime and waking salivary cortisol samples were collected. Analytes were assayed using a variety of commercially-available single-plex enzyme-linked immunosorbent assays.

At the time of the interim data review, 79 subjects had evaluable clinical rating scale data from the study baseline assessment (low GAF n = 25, high GAF n = 54), 46 of whom had biomarker data. These subjects' 15-analyte biomarker data were submitted to an unsupervised (K-means) clustering analysis to identify whether dissociable "biotypes" could be identified. Class membership in the two identified biotypes was compared against GAF group class label using Fisher's Exact Test.

**Results:** The two GAF groups were similar with respect to age, sex, and IQ. The low GAF group presented with poorer sleep (PROMIS-Sleep raw score for low GAF 21.4 + 8.34 vs. high GAF 16.3 + 6.65), more severe mania and depression (LIFE-PSR % weeks score > 3 for MDE: low GAF = 23.4% + 37.75 vs. high GAF = 4.7% + 15.27, Mania/hypomania: low GAF = 4.2% + 20.41 vs. high GAF = 0.4% + 2.64), and higher anxiety (GAD-7 score for low GAF = 7.7 + 5.31 vs. high GAF = 2.5 + 3.34). This group also reported higher use of concomitant antidepressant and anxiolytic medications (low GAF = 16.0% vs. high GAF = 9.3%).

Data-driven clustering of blood and saliva biomarkers identified two unique patient clusters (i.e., biotypes). One biotype was predominantly composed of individuals with high GAF scores, while the other biotype was predominantly composed of those with low GAF ($p < 0.002$). The biotype associated primarily with low GAF exhibited more severe prodromal mania and depression symptoms relative to the biotype associated with high GAF ($p <$

0.015). Analysis of the constituent analytes revealed that the two biotypes differed primarily in levels of kynurenine pathway metabolites, BDNF, ACTH, and serum cortisol.

**Conclusions:** Group differences at baseline cut across symptom domains, providing support to the poor specificity and heterogeneity of prodromal symptoms in high-risk subjects. Preliminary baseline results however indicate that, as expected, individuals with lower GAF scores were more psychiatrically symptomatic and had higher utilization of psychiatric medications. Significantly, humoral biomarker data can be used to identify individuals at risk for BD who exhibit poor global psychosocial functioning, and elevated prodromal mania and depression. Although still requiring replication in other cohorts, these findings provide biological support for the clinical distinction of high and low GAF groups defined in this study, and validate the use of circulating biomarkers in prediction of prodromal functioning in children at high risk of developing BD. When longitudinal data become available, an expanded dataset will assess whether clinical and humoral markers can be used for prodromal BD illness monitoring and prediction of conversion to MDD or BD.

**Keywords:** Bipolar Disorder, Personal and Social Performance (PSP) and Global Assessment of Functioning (GAF), Biomarker, Longitudinal Studies

**Disclosure:** Janssen Research and Development, LLC, Employee

## T115. A Double-Blind, Placebo-Controlled, Multicenter Study of Sirukumab as Adjunctive Treatment to a Monoaminergic Antidepressant in Adults With Major Depressive Disorder

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**Background:** Major Depressive Disorder (MDD) is a heterogeneous illness, and approximately one-third of depressed patients who are deemed to be treatment resistant may have distinct mechanisms underlying their depression, including systemic inflammation, resulting in reduced responsiveness to traditional monoamine treatment approaches.

Interleukin-6 (IL-6) is a cytokine known for its pleiotropic and proinflammatory functions which has been shown to be elevated in clinical depression in several meta-analyses; recent evidence suggests that elevation of IL-6 may be a marker of treatment non-response in MDD subjects. A putative role for IL-6 in the pathophysiology of MDD has been also suggested by preclinical data, which show a link between stress-induced depressive (i.e., anhedonia)-like behaviors and peripheral elevation of IL-6; notably, blockade of IL-6 in the periphery by neutralizing antibodies is able to prevent the development of a depression-like phenotype under social defeat stress, supporting the hypothesis that neutralizing IL-6 may have antidepressant therapeutic potential.

Sirukumab is a human immunoglobulin G1κ monoclonal antibody which binds to and neutralizes human IL-6. Post-hoc analyses of trials with monoclonal antibodies against IL-6 in non-psychiatric inflammatory conditions show a potential beneficial effect against the core symptoms of depression (depressed mood, anhedonia), which appears at least partially independent from the clinical benefits in the primary medical condition. However, whether sirukumab exerts antidepressants effects also in MDD subjects with no primary inflammatory condition is unknown.

**Methods:** The CNTO136MDD2001 study investigated the efficacy of sirukumab as adjunctive treatment to antidepressant therapy where sirukumab is compared to adjunctive placebo based on the total score change on the Hamilton Depression Rating Scale (HDRS17) from baseline to 12-week endpoint. All subjects were diagnosed with MDD and have had a suboptimal response to the current standard oral antidepressant therapy and a screening high sensitivity C-Reactive Protein (hsCRP) ≥ 3.00 mg/L. To enable investigation of the relationship between inflammatory biomarkers and clinical response, 50 subjects with hsCRP < 3.00 mg/L were also randomized to sirukumab or placebo but were not included in the primary efficacy analysis.

hsCRP was chosen as a potential biomarker to identify MDD subjects with an inflammatory component and who may be more prone to respond to sirukumab, as its synthesis is induced by IL-6 and elevated CRP levels previously were associated with greater responsiveness to the anti-TNF monoclonal antibody, infliximab, in MDD subjects.

Subjects were continued on their current monoaminergic antidepressant and received 50 mg sirukumab or placebo as a subcutaneous injection at Day 1, Day 28 and Day 56 during the 12-week double-blind treatment period.

**Results:** The study was conducted in 5 countries from August 2015 to May 2018. 193 subjects were enrolled in the trial and, of those, 142 subjects had hsCRP ≥ 3.00 mg/L.

Results for the change in HDRS17 total score at Week 12 based on subjects with hsCRP ≥ 3.00 mg/L at screening and baseline in the primary analysis did not favor sirukumab over placebo. Based on a MMRM model, the least-square mean difference (SE) between sirukumab and placebo was -0.8 (1.67). The difference between treatment groups was not statistically significant (one-sided $p = 0.310$). At Week 12, response and remission rates were similar for placebo and sirukumab.

Safety results were consistent with previous findings from other studies investigating sirukumab in patients with immunological conditions. Exploratory biomarkers analyses suggest that a higher baseline hsCRP threshold may be associated with greater drug separation vs placebo; however, those results were not consistent with the corresponding data for screening hsCRP values obtained 4 earlier. Several subjects enrolled in the study showed considerable variability of hsCRP between those two time points, questioning the utility of hsCRP as a putative treatment response biomarker.

Sirukumab was more effective than placebo in decreasing anhedonia symptoms measured with the SHAPS at week 12 (LS mean difference = 3.0; one-sided $p = 0.014$); greater clinical effect was seen with increasing baseline hsCRP levels.

**Conclusions:** The data from this study confirm the fact that a significant proportion of MDD subjects with suboptimal response to antidepressants display peripheral immune dysregulation. Neutralizing IL-6 using sirukumab was not effective at reducing conventional depression rating scale scores in subjects with suboptimal response to oral antidepressant and evidence of systemic inflammation as defined using hsCRP ≥ 3.0 mg/L. In contrast, post hoc tests suggested a beneficial effect on ratings of anhedonia, which merits further investigation. While it remains possible that drugs that target different peripheral or central immune targets may be effective in this population for improving conventional ratings of depressed mood, it also is conceivable that anti-inflammatory effects may more specifically act on the anhedonia symptom domain. In either case, further work is needed to discover more stable biomarkers or biomarkers combinations that may identify MDD subjects non-responsive to conventional antidepressants who show chronic low-grade inflammation and who may respond to novel treatments.

**Keywords:** Depression Inflammation Cytokine, CRP, Treatment Resistant Depression, Inflammation

**Disclosure:** Stock Shareholder, Employee

## T116. Repeated Intranasal Esketamine Administration and the BDNF SNP rs6265 are Not Associated With Post-Treatment Serum BDNF Levels in Adults With Treatment Resistant Depression

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**Background:** Esketamine is the S-enantiomer of ketamine, a N-methyl-D-aspartate receptor (NMDAR) antagonist that has demonstrated efficacy in rapidly reducing depressive symptoms of patients with Treatment Resistant Depression (TRD); this effect persists for several days beyond initial administration. A hypothesized mechanism for the persistence of ketamine's effects is by increasing AMPA receptor transmission, leading to increased release of neurotrophic proteins such as Brain Derived Neurotrophic Factor (BDNF) and in synaptic plasticity changes in the hippocampus and medial prefrontal cortex. Low levels of BDNF have been associated with Major Depressive Disorder and increases in peripheral BDNF have been observed following conventional antidepressant (AD) therapy. In rodent models, raising BDNF levels in the hippocampus improves depression-like behaviors, whereas increasing BDNF in the striatum appears depressiogenic.

Clinical trials reported a rapid increase in plasma BDNF measured at 4 h after a single infusion of ketamine, which negatively correlated with Montgomery Asberg Depression Rating Scale (MADRS) scores. Moreover, a Single Nucleotide Polymorphism (SNP) in BDNF, rs6265, which encodes an amino acid substitution of Val66Met, has been associated with BDNF levels and clinical response to a single infusion of ketamine.

Studies of ketamine's effects on peripheral BDNF levels have focused on acute time points following a single dose. Here we investigate whether an elevation in BDNF may be evident following multiple esketamine + AD treatments in serum sampled on Day 25 (3 days after the last esketamine administration). We also test a previously reported association of the rs6265 gene variant with clinical outcome in two larger studies following repeat dosing with esketamine + AD.

**Methods:** The Phase 3 trials, ESKETINTRD3001 (ESK3001, N = 346) and ESKETINTRD3002 (ESK3002, N = 237), were randomized, double-blind (DB) studies evaluating the efficacy, safety, and tolerability of fixed (ESK3001) and flexible (ESK3002) doses of intranasal esketamine plus an oral AD in adults with TRD (i.e. non-response to > 2 antidepressants). Eligible patients were randomized to either placebo (PBO) or esketamine (ESK3001: 56 mg or 84 mg; ESK3002: 56/84 mg); all participants also initiated a new, open-label, oral AD. Depression severity was measured by MADRS. Biomarker samples were taken at BL (pre-dose) and on Day 25 (D25). Serum BDNF was measured using a Meso Scale ELISA assay.

General linear modeling (GLM) was used to identify the relationship of BL BDNF levels to BL MADRS and a change in MADRS (dMADRS) at the end of DB (D28). Change in serum BDNF (dBDNF) at D25 from BL was calculated as a percentage of BL levels. A t-test was used to identify differences in dBDNF between PBO and esketamine-treated patients. Differences in dBDNF were further explored by splitting treatment groups by clinical response (greater than 50% reduction in MADRS from BL). GLM was used to identify correlations between rs6265 and BL BDNF and dMADRS at D2 and D28. Significance was defined as $p < 0.05$, uncorrected for multiple testing, for all analyses.

**Results:** Baseline levels of BDNF were not correlated with BL MADRS in either ESK3001 ($p = 0.78$) or ESK3002 ($p = 0.84$). A significant correlation between BL BDNF levels and D28 dMADRS was observed for the AD + PBO cohort in ESK3002 and the esketamine 56 mg + AD cohort in ESK3001, but not other groups (ESK3001: AD + PBO $p = 0.11$, ESK 56 mg + AD $p = 0.02$, ESK 84 mg + AD $p = 0.15$; ESK3002: AD + PBO $p = 0.01$, ESK + AD $p = 0.22$). No significant change in BDNF level between BL and D25 was evident in any group, and no difference was observed in dBDNF between AD + PBO and esketamine + AD groups in ESK3001 (ESK 56 mg + AD $p = 0.26$, ESK 84 mg + AD $p = 0.22$) or ESK3002 ($p = 0.98$). Furthermore, no significant relationships were observed when treatment groups were stratified by response at D28 ($p > 0.05$ for all tests). SNP rs6265 was not correlated with BL BDNF, dBDNF, or dMADRS at D2 or D28 ($p > 0.05$ for both trials, all treatment groups).

**Conclusions:** In these biomarker samples from participants with TRD, no significant correlation was found between BDNF and depression severity at BL. A significant correlation between BL BDNF and Day 28 dMADRS was observed in the ESK3001 ESK 56 mg + AD cohort; however, a significant correlation was also observed in the ESK3002 AD + PBO cohort suggesting that the effect is not specific to esketamine + AD treatment. Serum BDNF levels did not change significantly after repeat dosing of esketamine + AD at D25. Moreover, there was no correlation of the rs6265 SNP with serum BDNF levels or with clinical response to esketamine + AD.

Our studies do not exclude the possibility that acute, transient increases in BDNF may have occurred following esketamine + AD treatment; we did not measure BDNF within hours of treatment, as was performed in the previous positive studies with ketamine. Additionally, the literature is in disagreement as to whether peripheral and central BDNF levels are correlated, therefore our serum measures may not have direct relationship to BDNF release in specific brain regions. Nevertheless, our results based on 2 large clinical trials did not detect a persistent change in serum BDNF levels after repeated dosing with esketamine + AD. Finally, in this large sample, the rs6265 polymorphism did not significantly correlate with the clinical response to esketamine + AD following acute or repeated administration.

**Keywords:** BDNF, Intranasal Esketamine, BDNF Val66Met, Treatment Resistant Depression

**Disclosure:** Johnson & Johnson, Employee, Illumina, Employee (Spouse)

## T117. Intranasal Ketamine for Acute Suicide Ideation: A Trans-Diagnostic Approach: A Double-Blind, Randomized, Placebo-Controlled, Proof-Of-Concept Trial

Abstract not included.

## T118. ASPIRE 1 and 2: Phase 3 Randomized Studies of Esketamine Nasal Spray for the Rapid Reduction of Major Depressive Disorder Symptoms, Including Suicidal Ideation, in Adult Patients Assessed to be at Imminent Risk for Suicide

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**Background:** Major depressive disorder (MDD) is associated with an increased risk of suicide-related mortality. Esketamine (ESK) nasal spray is currently being developed for the indication of MDD with imminent risk for suicide. The initial proof-of-concept study of ESK nasal spray plus comprehensive standard-of-care (SoC) treatment has shown evidence of rapid improvement in

depressive symptoms, including suicidal ideation (SI), among patients with MDD at imminent risk for suicide. Currently, phase 3 studies are being conducted to further evaluate the efficacy and safety of ESK nasal spray + SoC vs placebo nasal spray + SoC in patients with MDD at imminent risk for suicide.

**Methods:** ASPIRE-1 and 2 are ongoing double-blind (DB), randomized, placebo-controlled, global, phase 3 studies being conducted in adult patients (aged 18-64 years) who have active SI and intent, and for whom psychiatric hospitalization is clinically warranted. Eligible patients (n = 224 in each study) are randomized (1:1) to ESK 84 mg or placebo twice a week for 4 weeks (Days 1, 4, 8, 11, 15, 18, 22, and 25 of DB phase) along with SoC antidepressants. The primary endpoint is change from baseline in the Montgomery-Asberg Depression Rating Scale (MADRS) total score at 24 h post first dose. The key secondary endpoint is change from baseline in the Clinical Global Impression of Severity of Suicidality Revised (CGI-SS-R) from the Suicidal Ideation and Behavior Assessment Tool (SIBAT) at 24 h post first dose. These and other efficacy outcomes, including remission (MADRS total score ≤ 12), resolution of suicidality (proportion of patients achieving CGI-SS-R score of 0 or 1) and change from baseline in Clinical Global Impression – Imminent Suicide Risk (CGI-SR-I) from SIBAT, are also evaluated as early as 4 h post first dose and through the day 25 DB endpoint. The patient-reported outcomes, including change from baseline in Beck Hopelessness Scale (BHS), European Quality of Life (EuroQol) Group, 5-Dimension, 5-Level (EQ-5Q-5L), Quality of Life in Depression Scale (QLDS), 9-item Treatment Satisfaction Questionnaire for Medication (TQSM-9) and patient-reported frequency of suicidal thinking from SIBAT, are evaluated through day 25 DB endpoint. Safety evaluations include treatment-emergent adverse events, transient sensory perceptual effects assessed by the Clinician-Administered Dissociative States Scale (CADSS), Modified Observer's Assessment of Alertness/sedation (MOAA/S), and suicidal thinking and behavior using SIBAT.

**Results:** The studies are being conducted in North America, South America, Europe and Asia and currently in open enrollment. A detailed description of the studies' design, patient demographics, and update on enrollment status will be provided.

**Conclusions:** These two clinical trials are intended to provide further evidence of efficacy of ESK nasal spray in the rapid reduction of depressive symptoms, including SI in these vulnerable patients. Findings of these studies are expected to be available in 2019. If positive, these results may support the indication of the first treatment for patients with MDD at imminent risk for suicide.

**Keywords:** Intranasal Esketamine, Major Depressive Disorder (MDD), Suicidal ideation

**Disclosure:** Janssen Research & Development, LLC, Employee

### T119. The Impact of Midazolam Versus Saline in Controlled Studies of Ketamine for Mood Disorders

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**Background:** Ketamine has emerged as the prototypical rapid-acting antidepressant, yet several issues impede its progress in both research and clinical domains. Among these problems is the integrity of the blind when ketamine is evaluated in randomized controlled trials. Due to the potent psychoactive effects of ketamine, there is concern that both patients and raters may be functionally unblinded when saline is used as the comparator. Murrough et al. (2013) published the results of the first trial of ketamine using midazolam (a benzodiazepine) as an active comparator in an attempt to improve the integrity of the blind. Unfortunately, because most ketamine trials have not formally evaluated whether the integrity of the blind is maintained, it is difficult to evaluate whether midazolam is an improvement over saline in this respect. Here, we evaluate the performance of midazolam as an active comparator by examining the effect size of controlled studies of intravenous ketamine using midazolam versus saline.

**Methods:** Patients were categorized into one of four groups, based on the condition to which they were assigned: 1) ketamine (midazolam), used in studies with midazolam as a comparator; 2) ketamine (saline), used in studies with saline as a comparator; 3) midazolam; and 4) saline. In all studies, the ketamine dose of 0.5-0.54 mg/kg was administered over 30-40 min. The midazolam dose was 0.045 mg/kg in one trial, and 0.02 mg/kg in two other trials. We compared clinical outcomes at Day 1 post-infusion, using a linear mixed model with a repeated effect of time and a random effect of study. The difference between treatment groups was evaluated with a series of between-group contrasts of improvement from baseline to Day 1.

**Results:** We obtained participant-level data from k = 9 studies (N = 367 subjects with Major Depressive Disorder or Bipolar Disorder; n = 106 participants in ketamine (midazolam), n = 81 in ketamine (saline), n = 83 in Midazolam, and n = 97 in Saline). By 24 h post-infusion, the improvement observed in ketamine (midazolam) (model-estimated mean improvement = 13.6, SE = 0.9) was greater than the improvement observed in the midazolam group (mean = 7.0, SE = 1.0) ($t360 = 4.9$, $p < .0001$), and the improvement observed in ketamine (saline) exceeded that of saline (mean = 1.9, SE = 0.9) ($t360 = 7.63$, $p < .0001$). The effect size for difference in change from baseline to Day 1 between ketamine (midazolam) and midazolam was d = 0.5 (95% CI: 0.3 – 0.7). For ketamine (saline) versus saline, the effect size was d = 0.8 (95% CI: 0.6 – 1.0). There was no difference between ketamine (midazolam) and ketamine (saline) in improvement; change under Midazolam significantly exceeded change under saline ($t360 = 3.7$, $p = 0.0002$).

**Conclusions:** The average effect of ketamine was smaller when compared with midazolam than when compared with saline, which was driven by greater improvement in the midazolam group compared to the saline group. An important limitation is that no trial directly compared midazolam to saline. One interpretation of these results is that midazolam was superior to saline in preserving the integrity of the blind. However, alternative explanations, such as the hypothesis that midazolam has antidepressant effects, cannot be excluded.

**Keywords:** Ketamine, Depression, Bipolar Depression, Clinical Trials

**Disclosure:** Janssen, Honoraria

### T120. A Biologically Realistic Computational Model of Visual Identification Reproduces Human Behavior of Ambiguous Face Emotion Interpretation

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**Background:** Affective psychopathology involving anger, anxiety, and depressed mood are associated with negative interpretation

biases of ambiguous social and emotional stimuli. These interpretation biases have been consistently measured with a number of types of stimuli: vignettes, social scenes, and facial affect. Of note, the latter has recently been translated into investigational treatments for disruptive mood dysregulation, conduct, depressive, and anxiety disorders. Despite the rapid clinical translation of this cognitive phenomenon, the neural basis of interpretive biases is unknown. It likely involves a distributed neural system, for which computational models are well suited to elucidating plausible mechanisms from neurons to behavior. Ultimately, our goal is to understand the neural mechanisms of this phenomenon to guide theory and focus further clinical translation. As a first step, we developed a biologically realistic computational model of face-emotion visual identification and evaluated its behavior when interpreting ambiguous facial expressions.

**Methods:** This study was conducted in two phases. The first phase established core human behaviors during two-alternative forced judgments of happy/angry face emotions. We used a task commonly used in research on psychopathology, the Interpretation Bias Task. We recruited an online sample of $N = 394$ adults, 21-76 years old, $M(SD) = 40.58(11.30)$ years completing IBT. Participants judged as happy or angry pictures of face emotions on a 15-interval linear morph continuum from 100% happy to angry. Three replicates of each face emotion on the continuum were presented at random for 200 ms, followed by a 150 ms visual mask, and then a response prompt until response. Sixteen happy to angry morph continua were created from the IAS Lab Face Set, and each participant judged just one of the 16 sets. The probability of an angry judgment on morphs ordered from happy to angry were modelled with a logistic curve. This measured the inverse temperature, the sensitivity to differences face emotion expression information on judgment. The reaction time cost for judgments of ambiguous face emotions (ambiguity cost) was calculated as difference between the mean reaction time of judgments for the 3 ambiguous morphs and the mean reaction time of the 4 most overt morphs, i.e. the two morphs on either end of the continuum.

The second phase of the study developed, trained and tested the Leabra Visual system (LVis) model in emergent (version 8.5.6) for face emotion identification. LVis is a biologically realistic, connectionist model representing neural populations in visual, temporal, and inferior frontal cortical areas. It was trained with 64 images of 100% happy or angry face emotions from the IAS Lab Face Set until it identified the emotion of the faces with 95% accuracy. In a test set of 16 face-emotion continua, we measured the inverse temperature of its judgments and ambiguity cost in CPU cycles in 11 of 16 face sets.

**Results:** In the first phase of the study, participants demonstrated a high inverse temperature $M(SD) = 5(6.64)$, $t(393) = 15.88$, $p < .001$. At the mean inverse temperature, 5, the probability of making the "wrong" judgment just one morph from the most ambiguous morph on either side is < 10-2. This suggests that people have a high sensitivity to small differences in happy or angry information in a morph, e.g. the logistic function formed a stair step rather than a smooth s about the most ambiguous morph. Ambiguity cost was $M(SD) = 100.37(139.46)$, $t(15) = 14.29$, $p < .001$. In the second phase of the study, LVis also demonstrated a high inverse temperature $M(SD) = 7.61(6.30)$, $t(15) = 4.84$, $p < .001$ and an ambiguity cost $M(SD) = 0.76(0.83)$ cycles, $t(10) = 3.0$, $p = 0.013$.

**Conclusions:** These results suggest that the visual system and early linguistic cortex are sufficient to produce a high sensitivity to small differences in face emotion information. Interestingly, slower processing of ambiguous information was also present. Slowing during identification of ambiguous information may a recruitment signal for control processes. For both phenomena, LVis suggests attractor dynamics as a core mechanism. An attractor state- such as setting on a happy or angry interpretation- may be induced by sparse information. Conflicting information, as in the case of ambiguity here, results in a longer time in which to settle into one attractor state. Further development of this model will involve simulating mechanisms of biases in face emotion perception by manipulating the strengths of the attractor states. This model may be used for simulating and identifying mechanisms for face-emotion perception, attention, and identification processing impairments commonly associated with affective psychopathology.

**Keywords:** Interpretation Bias, Computational Modeling, Mood Disorders

**Disclosure:** Nothing to disclose.

## T121. Development of a Touchscreen-Based Flanker Task in Rats for Translational Studies of Cognitive Control

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**Background:** Deficits in cognitive function, such as reward sensitivity and cognitive control, are a common feature of virtually all neuropsychiatric disorders. While perturbations in cognitive control have been studied extensively in humans, it has been challenging to develop rodent-based tasks to assess these complex processes and, consequently, to identify and advance innovative treatments for neuropsychiatric disorders. As part of a larger effort to create reliable and valid cross-species assays of cognitive function, we have developed a rodent version of the Eriksen Flanker Task to assess behavioral and neurophysiological indices of cognitive control.

**Methods:** Using fading and correction procedures combined with touch-sensitive response technology, we trained male and female Long Evans rats to discriminate between several distinct pairs of visual stimuli. Rats were trained in daily sessions with 100 trials in which each correct response resulted in a 30% sweetened condensed milk reward (0.1 ml). Discrimination was deemed successful when the criterion of 70% response accuracy was observed on two consecutive days in the final stage of the fading procedure. The results of validation studies indicated that detailed photographic stimuli (green leaf/violet flower) yielded appropriate stimulus control and were suitable stimuli for use in the Flanker Task. Following training under these final conditions, rats were surgically implanted with skull surface and depth electrodes, and neurophysiological data were collected during Flanker Task testing. In parallel studies, EEG data were collected from human subjects using the stimuli validated in the rodent task.

**Results:** All human subjects showed the expected Flanker interference effects of reduced accuracy ($p < 0.001$) and increased response latency ($p < 0.001$) on incongruent trial types. In addition, robust N200 components ($p < 0.001$) as well as increased theta power ($p < 0.001$) were noted when comparing incongruent and congruent trial types as well as increased error-related negativity (ERN; $p < 0.001$) and theta power ($p < 0.001$) for incorrect vs. correct responses. These results indicate that the stimuli chosen for the rodent Flanker Task elicit the expected effects in humans. All rodents tested show the Flanker interference effect of reduced accuracy on incongruent trial types ($p < 0.01$). Preliminary electrophysiological recordings in rodents suggest ERP and spectral findings were qualitatively similar to those observed in humans.

**Conclusions:** We have developed a touchscreen-based rodent flanker task that shows cross-species similarity in behavioral performance. Initial electrophysiological analysis in rodents indicates that ERP and spectral findings are similar to their well-characterized effects in human subjects. Ultimately, cross-species comparisons of behavioral and neurophysiological indices of cognitive control will afford new opportunities to evaluate potential therapeutics for a range of neuropsychiatric disorders.

**Keywords:** Flanker Task, EEG/ERP Electrophysiology, Touchscreen

**Disclosure:** Nothing to disclose.

### T122. Use of Psychotrophic Medication and Suicide Mortality in Bipolar Disorder

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**Background:** Suicide is one of the most common causes of death in bipolar disorder. In the recent years, use of antidepressants and anticonvulsants has increased on the expense of lithium in the treatment of this disorder. Not much "real-world" data from large inclusive cohorts is available to determine whether this switch in treatment preference could affect risk of suicide in bipolar patients. We aimed to study the comparative effectiveness of pharmacological treatments on suicide mortality in a nationwide cohort of Finnish patients with bipolar disorder ($n = 18{,}018$).

**Methods:** We studied the risk of suicide mortality during 1996–2012 among all patients who had been hospitalized due to bipolar disorder in Finland ($n = 18{,}018$; mean follow-up time 7.2 years) using prospectively gathered nationwide databases for hospitalization and dispensed medications. The primary analysis was a Cox proportional hazards model. Analyses were adjusted for the effects of time since diagnosis, order of treatments, current use of other treatments, polypharmacy within medication group, number of suicidal hospitalizations within a 2-year period (indicator of inherent risk of relapse), age at index date, sex, and calendar year of index date. Omission of the first 30 days from analysis after any switch in psychopharmacological treatment was used to control for protopathic bias. Results are reported as hazard ratios (HRs) with 95 % confidence intervals (95% CI).

**Results:** In comparison between use and no use among specific agents reaching nominal statistical significance, lithium (HR 0.33, 95% CI 0.24 to 0.47, $p < 0.0001$) and valproic acid (HR 0.61, 95% CI 0.48 to 0.79, $p = 0.00017$) were associated with the lowest risk of suicide as compared to non use in bipolar disorder (HR 0.30, 95% CI 0.20 to 0.44, $p < 0.0001$ and HR 0.52, 95% CI 0.39 to 0.71, $p < 0.0001$ respectively, when controlled for protopathic bias). On the other hand, use of antidepressants (HR 1.28, 95% CI 1.02 to 1.61, $p = 0.03$) were associated with a higher risk of dying by suicide as compared to non-use, although this result did not remain significant when controlled for protopathic bias (HR 1.13, 95% CI 0.87 to 1.46, $p = 0.36$). Lithium use was associated with a a 42 % lower risk for suicide mortality when compared with use of valproic acid (HR 0.58, 95% CI 0.39 to 0.86, $p < 0.007$).

**Conclusions:** Lithium and valproic acid should be considered as treatments of choice for patients with bipolar disorder who are at high risk for suicide. The increased risk associated with antidepressant use might in part or totally be due to confounding and bias, but it is obvious that lithium and valproic acid are associated with a substantially better outcome than antidepressants.

**Keywords:** Bipolar Disorder, Suicide, Lithium

**Disclosure:** Nothing to disclose.

### T123. Rare Variants are Associated With Antidepressant Remission

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**Background:** Approximately 12% of Americans currently take antidepressants, thus the ability to predict antidepressant drug response may have a large impact in public health. Rare genetic variants can contribute to 30-40% of functional variability in genes relevant to drug action. We investigated the hypothesis that rare functional genetic variants contribute to antidepressant drug response.

**Methods:** Mexican-Americans individuals with DSM-IV criteria for major depressive disorder participated in a prospective randomized double-blind pharmacogenetics study of 8 weeks treatment with desipramine or fluoxetine. The primary outcome measure was measured by the HAM-D. Whole exome genotyping data from 36 remitters and 29 non-responders were used for rare variant analysis and network and pathway analysis were performed with the list of significantly associated genes.

**Results:** We identified several genes significantly associated with treatment remission (FDR < 0.05), and their gene ontology (GO) processes included the involvement of sensory signaling pathway (olfactory and chemical sensory of smell), inflammatory processed (regulation of response to cytokine stimulus), nutrient transport (transport of sugars, bile salts and organic acids, metal ions and amine compounds), and meiotic cell cycle process. Desipramine- and fluoxetine-treated patients had no significant differences in demographic or baseline clinical characteristics.

**Conclusions:** These results indicate the involvement of rare functional variants in antidepressant drug response and the assumption that drug response phenotype has significant genetic heterogeneity.

**Keywords:** Major Depression Disorder, Pharmacogenetics, Antidepressants, Mexican Americans, Rare Variation

**Disclosure:** Nothing to disclose.

### T124. Lithium Response in Families Can Inform the Selection of Long-Term Treatment of Bipolar Disorder

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**Background:** Lithium is the first-line choice for recurrence prevention in bipolar disorder. Yet, it is fully effective in only about one-third of patients. Thus, it will be important to identify who might benefit from it and for whom lithium is not indicated. Several earlier studies suggested that response to long term lithium treatment runs in families. Such findings stimulated efforts to find genetic variants associated with good (poor) response and use these for individualized treatment selection. To overcome limitations of the earlier studies, small sample size in particular, we carried out a multicenter study and used a standardized assessment of treatment response.

**Methods:** We conducted a study in 4 collaborating centers: in Halifax (Canada), in two clinics in Cagliari (Italy) and in Poznan (Poland). There was a total of 75 families in which the proband

and at least one relative could be assessed for their response to long term lithium monotherapy. The relatives included 27 parents / children, 43 siblings, 9 second-degree and 7 third-degree relatives for a total of 86 proband-relative pairs. We compared the family data with those from 78 unrelated persons with bipolar disorder treated in a general psychiatry outpatient program. The response to treatment has been evaluated using a previously described and validated rating scale (Grof et al. J Clin Psychiatry 2002). The scale values range from 0 to 10 with scores of 7 or higher indicating good and scores of 0 to 6 poor / incomplete response. All four centers established very good inter-rater reliability previously (Manchia et al. PLoS ONE 2013).

**Results:** Among the relatives of lithium responders 68% were also good responders, while in relatives of non-responders, only 19% responded to treatment ($\chi2 = 19.8$, $df = 1$, $p < 0.0001$; tetrachoric correlation $r = 0.7$). The response rate in the unselected comparison group evaluated in the same way as the family sample was 30%, markedly lower than in the responders' relatives ($\chi2 = 18.3$, $df = 1$, $p < 0.0001$), but not significantly different from the rates in families of non-responders ($\chi2 = 1.3$, $df = 1$, $p = 0.26$). The odds of responding were 8.8 times higher in families of responders compared to non-responders (95% CI 3.2 to 24.3) and were even higher for the first degree-relatives only (OR = 10.4, 95% CI 3.2 to 33.4).

**Conclusions:** Our findings support the hypothesis of familial nature of response to lithium in bipolar disorder. Assessing the response in relatives can be time consuming and is not always possible. But when the information is available, family history of lithium response appears to be one of the strongest factors for individualizing long term treatment of bipolar disorder.

**Keywords:** Bipolar Disorder, Lithium, Long-Term Treatment, Personalized Medicine

**Disclosure:** Nothing to disclose.

### T125. Effect of Mu Opiate Receptor Gene Polymorphism, rs1799971 (A118G), on the Perceptual and Antidepressant Actions of Esketamine and Placebo

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**Background:** Preclinical data show mu-opioid receptor (MOPR, gene symbol: OPRM1) agonists produce antidepressant-like effects. However, ETS6103 (Tramadol (a MOPR agonist) controlled release) phase 2b trial failed to meet the primary endpoint of non-inferiority when compared to amitriptyline (ClinicalTrials.gov Id: NCT02014363). Moreover, low dose naltrexone (a MOPR antagonist) produced better antidepressant outcomes than placebo in patients on various antidepressant regimens (Mischoulon et al., 2017).

Ketamine, esketamine and arketamine are non-competitive, non-subtype selective, activity dependent open channel N-methyl-D-aspartate receptor (NMDAR) antagonists. Ki-values of displacing NMDAR ligand binding by ketamine and its more potent enantiomer, esketamine, are about 1 and 0.5 uM, respectively. These agents show weaker potency for MOPR with binding Ki-values of 28 and 11 uM for ketamine and esketamine, respectively. Williams et al reported (Society of Biological Psychiatry Annual Meeting, 2018) that naltrexone pretreatment blocked ketamine induced antidepressant effects (without affecting perceptual effects) in depressed participants, suggesting the hypothesis that ketamine's antidepressant effects were related to MOPR activity. Nevertheless, at the plasma concentrations achieved under antidepressant dose of esketamine, the MOPR occupancy would be lower than those expected to produce a pharmacodynamic effect.

The single nucleotide polymorphism (SNP) rs1799971, which involves a nucleotide substitution at position 118 (A118G) in OPRM1, resulting in a missense amino acid change (Asn44Asp) at a N-glycosylation site associated with decreased stability of the corresponding receptor protein, has been shown in clinical and preclinical models to attenuate antinociceptive and other responses to MOPR agonists. To explore the role of MOPR function on the antidepressant action of esketamine, we examined whether this loss-of-function genetic variant of OPRM1 diminished antidepressant effects of esketamine in participants with treatment resistant depression (TRD).

**Methods:** The clinical sample was drawn from a randomized, double-blind, active-controlled, multicenter study in male and female adult participants with TRD (NCT02418585). Subjects received flexible dosing intranasal esketamine (56 mg or 84 mg) or intranasal placebo twice per week plus a newly initiated oral antidepressant for 28 days. Montgomery–Åsberg Depression Rating Scale (MADRS) and Clinician Administered Dissociative States Scale (CADSS) were used as measures of depression severity and perceptual effects, respectively. Primary outcome measure was assessed by the difference in the change from baseline in MADRS total score between study arms at Day 28. Of 227 participants randomized, a total of 198 subjects were genotyped on the Illumina Omni2.5 M v1.3 array and imputed to the 1000 Genomes reference panel (phase 3, v5) using MaCH/minimac. The SNP of interest in this analysis, rs1799971, was imputed with high confidence (RSQ = 0.99). Post-hoc multiple linear regression (MLR) models were used to test for associations between MOPR SNP and change in MADRS at Day 2 and Day 28. Similarly, MLR models were used to test for association between MOPR SNP and peak responses on CADSS at Day 1 and Day 25 (assessed 40 minutes post-dose). All models were fit separately for subjects receiving esketamine and those receiving placebo, and were fit with and without correction for age, gender, BMI, 4 MDS components for ancestry, and baseline depression severity.

**Results:** Of randomized participants, 197 completed the double-blind period. Change in MADRS total score with esketamine nasal spray and oral antidepressant was superior to oral antidepressant and placebo nasal spray at Day 28 (LS mean [SE] difference vs. antidepressant plus placebo -4.0 [1.69], 95% CI: -7.31, -0.64; 1-sided $p = 0.010$); likewise, clinically meaningful improvement was observed with esketamine nasal spray plus oral antidepressant versus active control at earlier time points, including Day 2 (1-day post-treatment) (Popova V., et al. reported at the American Psychiatric Association Annual Meeting, 2018).

In the esketamine treated subjects, change from baseline in MADRS total score to Day 2 did not differ between participants with AA versus AG genotypes ($n = 101$, $p = 0.750$). On Day 28, participants with AG genotype showed a nonsignificant trend toward higher improvement relative to the AA participants ($n = 101$, $p = 0.09$). Trends in similar directions were observed in the Placebo group.

The effect of genotype on change in CADSS total score from baseline was not significant at either Day-1 or Day-25 in either participant group.

**Conclusions:** The rs1799971 (A118G) variant in OPRM1 did not show an esketamine-specific effect on either change in depression or dissociation ratings. At day 28, participants with AG genotype showed a nonsignificant trend towards improved MADRS change compared to AA patients under esketamine, opposite in direction from that predicted by the hypothesis that MOPR activation contributes to the antidepressant effect. Notably, Pecina et al (2015) reported that this polymorphism contributed to inter-individual variations in regulation of neurotransmitter responses during pain as well as under the placebo effect. Limitations of this

study include the current cohort's relatively small size for detecting minor effects and concomitant administration of oral antidepressant agents in both esketamine and placebo groups.

**Keywords:** Esketamine, Mu-Opioid Receptors, Depression, Antidepressant, Ketamine

**Disclosure:** Janssen Pharmaceutical Companies of Johnson and Johnson, Employee

### T126. Increased Transactive Response DNA-Binding Protein (TDP-43) Levels in Bipolar Disorder: A Postmortem Study

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**Background:** Frontotemporal dementia (FTD) is a progressive neurodegenerative disease that can be initially misdiagnosed as psychiatric illnesses, such as major depression and bipolar disorder (BD) due to the high prevalence of behavioral changes in its initial stages. Since BD patients presenting poor clinical outcomes show worse cognitive performances and lower hippocampal volumes, it has been proposed that a subgroup of BD patients may also present a progressive clinical pattern and neurodegeneration. Molecular mechanisms support this hypothesis, such as the accelerated epigenetic aging seen in BD patients. Abnormal aggregates of transactive response DNA-binding protein (TDP-43) are a major neuropathological marker in FTD. TDP-43 plays a role in dendritic local translation, affecting aberrant behaviors relevant to multiple psychiatric dimensions. Common clinical and biological mechanisms may interplay both, FTD and BD. We decided to investigate as to whether altered brain TDP-43 levels could also play a role in BD.

**Methods:** Post mortem brain samples were obtained from the Brain Bank of the Brazilian Aging Brain Study Group (BBBABSG). Diagnosis of BD was made using SCID through an interview conducted with an informant close to the deceased subject. This interview also collects sociodemographic data and evaluates the presence of neuropsychiatric symptoms (delusions, hallucinations, agitation/aggression, depression, anxiety, euphoria, apathy, disinhibition, irritability, motor disturbances, night behaviors and eating changes) three months prior to death using the Neuropsychiatric Inventory (NPI). The left hemisphere is fixed in formalin for morphological studies, while specific brain regions from the right hemisphere are frozen for biochemical analysis. For our study, we extracted total protein from frozen anterior hippocampus using Epiquik EpiQuik Whole Cell Extraction Kit (Epigentek, Farmingdale, NY, USA). TDP-43 levels were measured in the hippocampus using sandwich ELISA Human TDP-43 / TARDBP ELISA Kit (LS-F5278-Lifespan Biosciences, Seattle, Washington, EUA). To further investigate the role of TDP-43 in BD, we also measured the levels of brain-derived neurotrophic factor (BDNF) in the hippocampus of the same patients using human BDNF ELISA Kit (AB99978-Abcam, Cambridge, UK). For statistical analysis, between-group (BD versus non-psychiatric controls) differences were assessed using Exact fisher's test or Mann-Whitney test for categorical variables or continuous variables, respectively. Correlation analyses were conducted using Spearman's rho test. All brain samples were collected only after informed consent was obtained from a family member.

**Results:** We studied fifteen BD cases and eighteen normal controls. Cases and controls did not differ in age at death, sex, and schooling. The top three most frequent neuropsychiatric symptoms (through the neuropsychiatry inventory) observed in BD patients were irritability, agitation and depression (86.7%, 80% and 80%, respectively). We found higher TDP-43 levels in the hippocampus of BD, when compared to controls (Mann-Whitney Test, $p = 0.01$). Interestingly, a subgroup analysis including only BD subjects showed TDP-43 was negatively correlated with the total score of neuropsychiatric symptoms ($r = -0.623$*, $p = 0.017$). Likewise, and as expected, BDNF levels were also negatively correlated with the total score of neuropsychiatric symptoms ($r = 0.639286$, $p = 0.01$), although no significant differences regarding BDNF were found between cases and controls. Finally, TDP-43 and BDNF levels in the hippocampus of BD patients were positively correlated to each other ($r = 0.639286$, $p = 0.01$).

**Conclusions:** Our findings suggest TDP-43 may play a role on the biological pathways underlying BD. It is surprising, however, that neuropsychiatric symptoms were negatively correlated to TDP-43 levels. Since we found the same results for BDNF, and also a positive correlation between TDP-43 and BDNF levels, we hypothesize whether TDP-43 may have a protective effect on BD symptoms. Our study has important limitations, such as the small sample size, and lack of information concerning mood state at the time of death. Future studies should examine further the role of TDP-43 in the pathophysiology of BD.

**Keywords:** Postmortem Brain Tissue, Bipolar Disorder, Dementia, TDP-43

**Disclosure:** Nothing to disclose.

### T127. Prefrontal Glutamine Level and Risk for Major Depression: An MRS Study in Young Adults

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**Background:** There is growing evidence for glutamate dysfunction in the pathogenesis of major depressive disorder (MDD). It is important to study this pathology before the onset or in the early phases of the illness. New magnetic resonance spectroscopy (MRS) measures allow for determining glutamine, the principal metabolite of synaptic glutamate that is directly related to glutamate levels in the synaptic cleft.

**Methods:** In contrast to previous investigations, this study used community-based recruitment methods and a dimensional approach to depressive vulnerability. In the study protocol, neuroticism was defined as the primary outcome. We examined young adult participants recruited from the general population in a cross-sectional study using 3-T 1H-MRS in. The total sample of $N = 110$ (61 females) included 18 individuals suffering from MDD.

**Results:** We found that glutamine and glutamine-to-glutamate ratio were correlated with neuroticism in the whole sample ($p < 0.005$). Individuals with MDD tended to have higher glutamine levels than non-MDD individuals ($p = 0.078$). Increased glutamine levels were associated with amygdala activity while processing threat-related stimuli and with depression-related resting state connectivity. Lack of self-confidence and emotional instability were the clinical correlates of glutamate dysfunction. There was a trend towards a negative correlation between mature brain-derived neurotrophic factor (BDNF) and glutamine levels ($p = .064$).

**Conclusions:** This study suggests that an increased prefrontal glutamine level is a vulnerability factor for MDD. Further understanding of glutamate dysfunction in the early phases of depressive illness may lead to new therapeutic strategies to prevent and treat depression.

**Keywords:** Glutamine, Depression, Glutamate GABA, Neuroticism

**Disclosure:** Nothing to disclose.

### T128. Effect of Treatment Resistance on Whole Brain Voxel-Based Morphometry in Patients With Major Depressive Disorder

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**Background:** Treatment-resistant depression (TRD), defined as failure to respond to one or more antidepressant treatment trials in patients with major depressive disorder (MDD), represents a major public health problem. Prior work has investigated regional brain differences between patients with MDD and non-depressed individuals, however few studies have investigated patterns in brain structure specific to TRD.

**Methods:** We examined the relationship between brain structure and treatment-resistance in a sample of unmedicated patients with MDD (N = 61) and demographically similar healthy controls (N = 41). All subjects underwent neuroimaging with MRI at 3 T and current depression severity was measured using the Montgomery-Asberg Depression Rating Scale (MADRS). Patients were classified by treatment resistance according to the Maudsley Staging Model (MSM), which accounts for treatment trials, severity of illness, and duration of presenting episode. Whole-brain voxel-based morphometry (VBM) analysis was conducted for gray matter using Advanced Normalization Tools (ANTs). The model included age, sex, and MDD diagnosis as covariates, along with TRD status and number of past antidepressant medication failures. Multiple comparisons correction was implemented using non-parametric cluster correction via permutation and Markov Chain Monte Carlo estimation to set $p < .05$ for whole-brain analyses. Region of interest (ROI) analyses focusing on the anterior cingulate cortex (ACC), the hippocampus, and the amygdala as defined by the Brainnetome atlas were also conducted.

**Results:** Greater number of antidepressant failures was associated with decreased gray matter volume (GMV) in a cluster centered on the left thalamus (x = -12, y = -20, z = 2, k = 14,598 mm3) and extending into the hippocampus and fusiform gyrus, controlling for current symptom severity. Traditionally defined TRD (2 or more antidepressant medication failures) was associated similarly with decreased GMV in the fusiform gyrus (x = -36 y = -63, z = -17, k = 3,812 mm3). Conservatively defined TRD (MSM score > 2) was negatively associated with GMV in the right retrosplenial cortex and precuneus (x = 15, y = -55, z = 14, k = 693 mm3). Within the same model, MDD diagnosis was negative associated with GMV in the dorsolateral prefrontal cortex (x = -42, y = -45, z = 6, k = 732 mm3) compared to healthy controls. ROI analyses indicated a significant effect of TRD status in the left and right posterior hippocampi (p's < .01), controlling for MDD diagnosis and symptom severity.

**Conclusions:** In the current study including a total of N = 102 subjects, we found convergent evidence for an association between TRD status and reductions in gray matter volume within midline cortical and subcortical regions when controlling for age, sex, MDD diagnosis, and illness severity. Our study implicates thalamic and medial temporal regions including the hippocampus and fusiform gyrus, as well as cortical components of the default mode network including the retrosplenial cortex and precuneus, as abnormalities specific to TRD. These observed alternations may represent either a risk factor or a consequence of treatment resistance. Future prospective studies will be critical to delineate early brain markers with prognostic significance in patients before the development of TRD.

**Keywords:** Depression, Treatment Resistant Depression, Human Neuroimaging

**Disclosure:** Nothing to disclose.

### T129. Is Your Glass Half Empty or Half Full? Single Subject Prediction Based on Anticipatory Pain Response in Healthy and Depressed Adults

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**Background:** Anticipation is an inherent component of emotional processing. It shapes our experience to both rewarding and aversive stimuli. It has been suggested that there is individual variability in anticipatory response in that some individuals will always expect the worst. It is particularly relevant to those with depression where maladaptive anticipatory brain response has been consistently demonstrated. For example, during anticipation of pain, depressed individuals show reduced frontal control and increased emotional reactivity and activation within interoceptive and salience networks, a response that correlates with depressive symptoms severity (Strigo et al 2013). To date, anticipatory brain responses to aversive stimuli have mostly been examined using group-level predictions. Here we employ single-subject level predictions to determine individual subject's propensity to expect better or worse when anticipating experimental pain. Consistent with cognitive models of depression (Beck 1987), we hypothesized that when presented with an ambiguous situation where the intensity of the upcoming pain could either be low of high, those with depression would more often be labeled as anticipating high, i.e., worse pain.

**Methods:** A total of 52 participants were examined: 30 (15 F) young un-medicated adults with current Major Depressive Disorder (MDD) (age: 27.8 ± 7.9) and 22 (11 F) healthy subjects with no history of MDD (age: 26.8 ± 8.7). Groups did not differ significantly in age, race, gender or level of education. The Structured Clinical Interview for DSM-IV was administered to all participants. Functional MRI (fMRI) data were collected during an event-related experimental heat pain anticipation paradigm. Subjects were cued to anticipate heat pain of both known (high pain or low pain) and unknown (either high pain or low pain at 50% probability) intensities. All temperature stimuli were applied to each subject's left forearm using an MR-compatible thermode. For the purposes of this study, single-subject multi-voxel pattern analysis (MVPA) was used to identify an individual's (i.e., subject-specific) fMRI signatures of pain anticipation at two different levels, anticipating high-pain (HP) and anticipating low-pain(LP), which are made known to the individual during the experiment. Per subject, the fourteen training sets(HP and LP) were first input to the classifier to analyze the conditional differences. This allowed for the discrimination between each individual's neurobiological signature of high and low pain anticipation. In order to predict each participant's anticipation based on their known neurobiological signature of HP or LP, regression analysis of the regional mean activation was first completed by way of least absolute shrinkage and selection operator (LASSO). LASSO was performed on a single-subject basis, in which the training set was an individual's brain response during known anticipation trials (HP or LP), and the test set was the same individual's brain response during the unknown anticipation trials. Regression was first fit to the training set and then cross-validation of the generalized linear model was performed through glmnet prior to performing predictive analysis for the purposes of labeling individual unknown conditions. Predictions were made at a binary level

(HP vs. LP), based on correlation of the -coefficients to the cross-validated glmnet model. Output of the prediction analysis was either HP(1) or LP(0) for each unknown anticipation trial in each individual. To bias feature selection, and due to the pivotal role, that the insula plays in pain anticipation(Craig 2009) we limited brain regions to 6 insula ROIs on each side (Faillenot et al 2017), for a total of twelve regions: (1) posterior long gyrus, (2) anterior short gyrus, (3) middle short gyrus, (4) posterior short gyrus, (5) anterior inferior cortex, and (6) anterior long gyrus.

**Results:** First, we found that neural patterns of insula activation between low and high pain anticipation are reliably distinguishable on a single-subject level. Second, we found that across the entire sample, over 80% of unknown anticipatory conditions were labeled as HP, or expecting worse pain. Nevertheless, as hypothesized, significantly more cases were labeled as HP in the depressed than in the control group (chi = 3.9; $p < 0.05$). In the current sample, we found no significant relationship with the overall depression severity and anticipatory labeling. Finally, anterior short gyrus on the right side showed the highest deterministic beta coefficient in anticipatory predictions in our study.

**Conclusions:** We provide further support on the ability to reliably discriminate between anticipation of different levels of pain and extend it to single subject level discrimination. In particular, our results represent unique evidence supporting the predictive capacity of the insula parcellations in processing ambiguous cues related to impending pain on a single subject level. Importantly, these results are consistent with the increased propensity of those with depression to expect the worst, consistent with the "glass half-empty" philosophy. Importantly, our methodology shows great individual variability in response to pain anticipation peculiar to both healthy and depressed participants. Our current work is evaluating stability of our predictions using single-subject multi-session data and the role of these predictions in vulnerability for mental and physical illness.

**Keywords:** Machine Learning, Mental Pain, Insula, Interoception, Functional MRI (fMRI)

**Disclosure:** Nothing to disclose.

## T130. 'Inflammaging' Selectively Disrupts Structural and Functional Integrity of Subcortical Brain Structures to Precipitate Cognitive Impairments in Depression

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**Background:** Chronic depression is often associated with poor treatment response, cognitive decline and neuroprogression. Risk of neuroprogression in mood disordered subjects may be compounded by 'Inflammaging' - a state of sterile, chronic, low-grade inflammation seen during aging – also implicated in the pathogenesis of age-associated medical disorders and neurodegeneration. Monocyte chemoattractant protein (MCP)-1 - a chemokine that signals trafficking of peripheral immune cells to the site of inflammation - is emerging as a promising marker of inflammaging. The exaggerated release of MCP-1 by brain immune and glial cells is seen following their conversion to a senescence-associated secretory phenotype (SASP). Increased MCP-1 concentrations have also been reported in neuroinflammatory (HIV and multiple sclerosis), autoimmune (inflammatory bowel and rheumatoid diseases) and neuropsychiatric disorders (depression) and among the frail elderly. We hypothesized that greater increases in plasma MCP-1 will be seen among older subjects (in a cohort of depressed patients) enabling the use of age and MCP-1 to define inflammaging in depressed subjects. Building on previous data, we also hypothesized that inflammaging would be associated with a greater impact on corticostriatal brain systems resulting in functional changes.

**Methods:** 41 medication-free depressed subjects (per DSM-IV criteria) underwent blood and cerebrospinal fluid (CSF, n = 35) sampling for inflammatory markers along with structured behavioral and neurocognitive assessments as part of a larger NIH-funded study (NCT1426997). Scans were obtained using a Siemens-3T Trim Trio scanner to assess structural (T1-MPRAGE), chemical (magnetic resonance spectroscopy, MRS) and functional (resting-state functional MRI, rsfMRI) integrity of the brain. Participants' age and plasma MCP-1 were combined using a K-means clustering algorithm to identify two subgroups of depressed subjects with contrasting profiles ('inflammaging' group, n = 22, and comparison group, n = 20). Volumes of cortical and subcortical regions of interest (ROIs) were calculated using T1-MPRAGE segmentation using Desikan-Killiany atlas in FreeSurfer. Absolute concentrations of neural and glial metabolite markers were estimated using magnetic resonance spectroscopy (MRS) on the left basal ganglia region. Intrinsic neural activity in the same basal ganglia region was measured using Regional Homogeneity (ReHo) - a measure of concordance in spontaneous local brain oxygen dependent (BOLD) fluctuations between neighboring voxels in rsfMRI. Volumetric differences between the two groups-of-interest were examined after controlling for sex, BMI, race, and total brain volume and after application of false discovery rates (FDR)-correction to limit false-positive errors. The volume changes in ROIs were examined for associations with MRS, ReHo, and neurocognitive measures.

**Results:** As hypothesized age and MCP-1 were significantly associated (beta(b) = 0.40, p = 0.009, Cohen's d = 0.87). K-means clustering provided 2 subgroupings with significant differences - 'inflammaging' [n = 22, mean age(SD) = 43.9(10.5), mean MCP-1 (pg/ml) = 163.0(55.6)] and 'comparison' groups [n = 20, age = 31.8(7.7) MCP-1 = 94.9(17.1)] ($p < 0.001$ for both age and MCP-1 difference). CSF (but not plasma) measures of c-reactive protein (CRP, b = 0.31, FDRp = 0.03, d = 0.76), MCP-1(b = 0.39, FDRp = 0.03, d = 0.84) and soluble tumor necrosis factor receptor type 2 (sTNFR2, b = 0.42, p = 0.02, d = 0.91) were significantly elevated in the inflammaging subgroup. Inflammaging group status was associated with decreases in total gray matter volume (b = -0.26, FDRp = 0.02, Cohen's d = 0.73) along with substantial reductions in subcortical (SCgray) gray matter volume (b = -0.42, FDRp = 0.002, d = 1.01). Regional volume loss impacting bilateral caudate (left: b = -0.63, FDRp = 0.002, d = 0.70, and right: b = -0.60, FDRp = 0.003, d = 1.18) and bilateral putamen (left: b = -0.43, FDRp = 0.03, d = 0.50, and right: b = -0.53, FDRp = 0.007, d = 0.62) was also seen in association with inflammaging group status. The interaction between inflammaging and subcortical gray matter volume (SCgray*inflammaging) significantly predicted right medialorbitofrontal volume (b = 0.56, p = 0.02, d = 1.09) indicating an extension of inflammatory pathology into reward circuit structures. MRS measures of astroglial marker myo-inositol in the left basal ganglia predicted gray matter loss in the subcortex [b = -0.39, p = 0.01, d = 0.92] and the whole brain [b = -0.41, $p < 0.001$, d = 1.74]. Scgray decreases were associated with ReHo decreases in the left basal ganglia VOI used for MRS [b = 0.66, p = 0.02, d = 0.96]. Finally, SCgray*inflammaging group interaction predicted performance on executive function measured using 'Stockings of Cambridge' [b = -0.61, p = 0.006, d = 1.06] despite a comparable severity of depression in both groups.

**Conclusions:** Older age was associated with increases in MCP-1 in this depressed group further validating preclinical data. Gray matter loss in the subcortical region had functional consequences (decreased ReHo) and impaired performance in tests of executive function. Increased myo-inositol may signify activated SASP-astroglia and hence astroglial-stabilizing agents (such as riluzole)

previously demonstrated to reverse age-associated neural loss in preclinical models may be used to target brain volume loss in this group.

**Keywords:** Neuroimmunology, Aging, Magnetic Resonance Imaging, Major Depression

**Disclosure:** Nothing to disclose.

### T131. Hippocampal Subfields in Acute and Remitted Depression – a Longitudinal 7 T MRI Study

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**Background:** Alterations of total hippocampal volume as well as changes in hippocampal subfield volumes associated with stress and genetic susceptibility were suggested as disease and treatment response marker [1]. Moreover, studies demonstrated differences of specific hippocampal subfields (HS) in acute, remitted, geriatric or bipolar depression [2,3]. Investigations of hippocampal subfield dynamics are simplified by automated structural magnetic resonance imaging (sMRI)-based delineations. Using this approach, a cross-sectional treatment study with one measurement demonstrated increased hippocampal tail volume as correlate of remission in relation to healthy subjects, while other subfield regions exhibited alterations due to successful remission [4]. We aimed to reproduce these subfield volume dynamics in acute and remitted depression with a ultra-high field MRI-system with a longitudinal design and by including a stable remitted and a healthy control group. Accordingly, we expected lower subfield values in acute depressed patients and hypothesized increases in HS volumes after antidepressant treatment in relation to control groups

**Methods:** We recruited a total N of 70 subjects, 22 healthy controls (HC, age ± SD = 25.9 ± 6.7), 28 medication-free, remitted depressed subjects (rMD, 26.6 ± 5.8 remission stable at least 3 months prior to inclusion) and 20 acute depressed subjects with a major episode in MD (aMD, 30.4 ± 9.6). Acute depressed subjects underwent antidepressant treatment with escitalopram for at least 6 weeks, and, upon non-response, were switched to venlafaxine for a total treatment period of 12 weeks. All other subjects were pharmacologically untreated.

Each subject underwent two 7 T sMRI scans (Siemens Magnetom) with a 32-channel head coil applying a MP2RAGE sequence with TR = 4060 ms, TE = 3.02 ms, a scan-time of 11:18 min, in a FOV of 192 × 312 × 384 (x/y/z) resulting in a voxel size of: 0.74 × 0.68 × 0.68 mm (x/y/z). Hippocampal subfield segmentation was performed with FreeSurfer 6.0, all data were inspected and underwent automated and manual quality control.

We compared baseline and posttreatment hippocampal subfield volumes with a repeated measures ANOVA in R, adjusted for age, sex and total intracranial volume. Post-hoc group wise comparisons were conducted by estimated marginal means for interactions of the linear models within the ANOVAs, all p-values are reported at an $\alpha < 0.05$, multiple comparisons were corrected with the Tukey's range test, uncorrected p-values are reported as well.

To investigate association of subfield values' changes with remission, absolute subfield value changes were correlated with HAM-D changes.

**Results:** We detected a difference between groups ($F_{2,3213} = 17.15$, $p < 0.001$), and a trendwise interaction for group × timepoint ($F_{46,3213} = 2.99$, $p = 0.05$), while other relevant interactions such as group × region ($F_{46,3213} = 0.84$, $p = 0.74$), timepoint × region ($F_{23,3213} = 0.1$, $p = 0.999$) and group × timepoint × region ($F_{46,3213} = 0.1$, $p = 0.999$) were not significant. Specifically, the right hippocampal fissure (MRI-1: $t3213 = 3$, pTukey = 0.034, Cohen's $d = 0.11$; MRI-2: $t3213 = 2.4$, puncorr = 0.016, $d = 0.08$) and hippocampal-amygdalar-transition zone (MRI-1: $t3213 = 2.13$, puncorr = 0.034, $d = 0.07$; MRI-2: $t3213 = 3.2$, pTukey = 0.017, $d = 0.11$) exhibited larger values in rMD compared to HC, both at MRI-1 and MRI-2 cross-sectionally. Moreover, we detected significantly larger right subiculum values in aMD patients ($t3213 = 2.02$, puncorr = 0.044, $d = 0.07$) and rMD subjects ($t3213 = 2.14$, puncorr = 0.033, $d = 0.08$) compared to HC at MRI-2 only.

Significant positive correlations were obtained in right presubiculum ($r = 0.52$, $p = 0.02$) as well as a positive correlation in the left hippocampal tail, reaching significance at trend level ($r = 0.44$, $p = 0.054$). There were no significant positive correlations with HAM-D changes in any one of the subfields encompassing the dentate gyrus (all $p > 0.05$).

**Conclusions:** In a longitudinal analysis of hippocampal subfields in acute depressed, remitted depressed patients and healthy controls we found significant differences predominately in the right hippocampus in cross-sectional analyses only. Notably, in the hippocampal fissure, HATA and subiculum, patients in stable remission exhibited more gray matter than healthy controls. Cross-sectional differences match a previous study [4], while it is striking that we did not reproduce longitudinal changes upon antidepressant treatment with escitalopram and venlafaxine. This could be attributed to differential MRI-parameters (IP-SPGR sequence, 8-channel head coil, 3 T in Maller et al.), whereby our setup (MP2RAGE, 32-channel, 7 T) might have exhibited higher resolutions. Further studies with 7 T with larger and better age-balanced samples to detect smaller effects are warranted.

**Keywords:** Hippocampal Subfields, 7-Tesla, Depression, Longitudinal Imaging

**Disclosure:** Nothing to disclose.

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### T132. Age-Related Differences in Amygdala Activation During Face-Emotion Processing in Pediatric Bipolar Disorder and Familial Risk for Bipolar Disorder

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**Background:** Bipolar disorder (BD) is a severe and impairing clinical phenotype that is highly heritable, with estimates ranging from 59% to 85% (Lichtenstein et al., 2009; McGuffin et al., 2003). Thus, research on familial high-risk samples (i.e., participants who

have a first-degree relative with BD) is critical. Familial high-risk samples can help identify risk and resilience markers of BD by elucidating neurobiology that these individuals share and do not share with individuals with the diagnosis. Aberrant amygdala function during face-emotion processing has been proposed as a marker of genetic risk for BD (e.g., Brotman et al., 2010; Garrett & Chang, 2008; Manelis et al., 2015; Olsavsky et al., 2012; Rich et al., 2006). However, no study has investigated age-related developmental differences in this neural marker in pediatric BD and familial risk for BD. Age-of-onset-data suggest that BD typically emerges during the second or third decade of life (i.e., during or post-adolescence) (e.g., Leboyer et al., 2003; Kessler et al., 2005). Here, in two separate studies, we use two complementary paradigms to examine age-related differences in amygdala activation during face-emotion processing in a familial high-risk sample of early-to-post adolescent youth.

**Methods:** Study 1 recruited adolescents aged 12 to 21 years with BD ($n = 20$), at familial risk for BP (one first-degree relative with BD; at risk, AR; $n = 20$), and healthy volunteers (HV; $n = 28$). During fMRI scanning, participants judged the gender of happy, fearful, and angry face-emotions, each of which varied in intensity of expression (50%, 100%, 150%). Study 2 recruited adolescents aged 11 to 19 years at familial risk for BD (AR; $n = 21$) and healthy volunteers (HV; $n = 25$). During fMRI scanning, participants labeled the emotions of happy, fearful, and angry faces, each of which again varied in intensity (50%, 75%, 100%). Across both studies, groups were matched on age, IQ, and sex; the samples were partially overlapping across studies. In each study, blood-oxygen-level-dependent (BOLD) signals in the right and left amygdala were extracted as regions of interest. Multivariate model analyses in AFNI examined group, age, face-emotion, and face intensity as predictors of BOLD activity.

**Results:** In Study 1, a group-by-emotion-by-age interaction emerged in the left amygdala ($F(3.76,157.8) = 2.7$, $p = .04$). Post-hoc analyses revealed that AR youth exhibited increased activation to angry faces with advancing age ($p < .02$), whereas both BD and HV youth did not show this developmental pattern. In Study 2, a group-by-emotion-by-intensity-by-age interaction emerged in the left amygdala ($F(4, 344) = 7.27$, $p < .001$). Post-hoc comparisons suggested that the interaction was driven by increased activation with age to high intensity angry faces in FS ($p = .04$) youths relative to HV youth.

No interaction emerged in the right amygdala.

**Conclusions:** A similar pattern of findings emerged across two studies spanning implicit and explicit face-emotion processing in familial risk for BD. Youth at familial risk for BD uniquely showed increased activation with increasing age to (high intensity) angry expressions, relative to both youth with BD and healthy adolescents. Studying developmental change in risk correlates across adolescence to early adulthood is important, given that this developmental period is a time where BD often first manifests. Study 1 suggests that age-related increase in amygdala activation during face-emotion processing is associated with at-risk status, rather than with the illness itself. Replication with longitudinal samples is needed.

**Keywords:** Bipolar Disorder, Developmental Psychopathology, At-Risk

**Disclosure:** Nothing to disclose.

### T133. Cortisol Inhibition of Sadness-Induced Subgenual Cingulate Activity

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**Background:** Many previous studies have linked 1) cortisol secretion levels and 2) the sensitivity of the brain to cortisol to a diagnosis of major depression and to the severity of depressive symptoms. Cortisol sensitivity has also been shown to normalize with successful treatment of depression and to predict future relapse. While associations between cortisol and depression are commonly observed, insights into the direction of causality and the mechanisms of action behind these associations have been difficult to determine. In order to study the direction of causality and the potential mechanism of action of cortisol, we administered cortisol (oral hydrocortisone acetate) to individuals experiencing symptoms of depression and to healthy individuals. We then used functional magnetic resonance imaging (fMRI) to test the effects on sadness-induced subgenual cingulate activity.

**Methods:** In this preliminary analysis, 35 participants (27 healthy, 8 depressed) completed a double-blind placebo-controlled cross-over design study. 2 separate fMRI scanning sessions were conducted for each participant. One of the scanning sessions tested the subgenual response to sadness (induced by pictures and videos) before and after cortisol administration of 0.65 mg/kg. The other scanning session tested the same changes in the subgenual cingulate response that occurred after an identical placebo dose.

**Results:** Cortisol suppresses sadness-induced subgenual cingulate activity to a greater extent than placebo (Wilcoxon paired t-test median = -0.24, 95% CI -0.38-0.04, $p = 0.046$). This finding is driven by strong cortisol suppression of subgenual activity in healthy participants, and a blunted suppression in participants with depression. Across all participants, the magnitude of cortisol's ability to suppression the subgenual cingulate is correlated (Spearman's $r = 0.35$, $p = 0.025$) with depression symptom severity (as measured by BDI-II). The higher the depressive symptoms the less cortisol is able to suppress sadness induced subgenual cingulate activity.

**Conclusions:** The sensitivity of the brain to cortisol has been strongly associated with symptoms of depression. However, the direction of causality and the mechanism of action behind these associations has proven elusive. In this preliminary analysis, we demonstrate that 1) cortisol has a suppressive effect on sadness-induce subgenual cingulate activity and 2) that a reduced cortisol suppression of subgenual cingulate is associated with higher symptom severity. These results suggest that an acquired insensitivity to cortisol could be diminishing the effectiveness of cortisol's ability to suppress the subgenual response to sadness and thus causing the expression of a pathological version of normal sadness in patients with depression.

**Keywords:** Cortisol, Depression, Subgenual Cingulate Cortex, Emotion

**Disclosure:** Nothing to disclose.

### T134. Examining Factors Leading to Underutilization of Electroconvulsive Therapy in the United States

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**Background:** Electroconvulsive therapy remains the gold-standard treatment for severe depression, yet it is often underutilized. While various factors likely contribute to this, there has been no systematic evaluation of factors leading to underutilization. Here, we use a mixed-methods approach to explore provider-level characteristics that may be associated with underutilization of ECT and geographic variation in rates of use.

**Methods:** First, we identified ECT providers from the Centers for Medicare and Medicaid Services database of Provider Utilization and Payment Data in 2015. This allows for the extraction of provider data on any physician who has provided a billable procedure to 10 or more Medicare patients. We utilized the Current Procedural Terminology (CPT) code 90870 to identify those who provided ECT to Medicare patients in 2015. We examined geographic variability in the number of ECT providers and ECT centers per capita identified through this database. We correlated this with previous data from a commercial claims database representing 47 million patients on the number of ECT per total patients with a diagnosis of Major Depressive Disorder (MDD) within each geographic region. Finally, qualitative methods were used to conduct in-depth interviews with a subset of 16 ECT providers to identify key factors that may preclude the initiation or expansion of ECT services.

**Results:** We identified 690 physicians who were part of approximately 477 ECT centers that provided ECT services to 10 or more Medicare patients in 2015 in the United States. There was substantial geographic variation in the number of ECT providers per capita (using 2010 census data), ranging from 12.8 ECT providers per million capita in Maine to no ECT providers in Alaska, Idaho, and Nevada. The mean number of ECT providers per million capita per state was 3.01 (SD 2.64). The mean number of ECT centers per million capita per state was 2.09 (SD 1.87), with a median of 1.42 and a range of 0 to 9.8. Expectedly, there was a statistically significant correlation between the number of providers per million capita per state and the proportion of patients with Major Depressive Disorder who received ECT in a commercial claims database in a given state (adjusted $r2 = 0.21$, $p < 0.01$); this was also true for the number of ECT centers per million capita per state (adjusted $r2 = 0.20$, $p < 0.01$). Preliminary thematic analysis of the interviews with 16 ECT providers identified several factors that preclude the initiation of new ECT centers and expansion of existing ECT centers. Most prominent among these factors included lack of comparable financial compensation when competing against other medical procedures for space and clinical support resources, stigma and lack of knowledge on the part of medical providers (nursing, anesthesia, and potential referring psychiatrists), and lack of support from administrative leadership. Notably, unwillingness to undergo the procedure and stigma on the part of the prospective ECT patient did not feature prominently from among these factors.

**Conclusions:** Wide geographic variation in the density of ECT providers and services was observed within the United States. While it was expected that there was a correlation between the density of ECT providers/centers and proportion of patients with MDD who were treated with ECT, the density of providers/centers only accounted for 20-21% of the variability in geographic variation of ECT utilization rates. Other local factors, as identified in the qualitative analysis, may also substantially contribute to ECT utilization rates.

**Keywords:** Electroconvulsive Therapy, Treatment Access, Mood Disorders

**Disclosure:** Nothing to disclose.

### T135. Novel Transcriptome-Based Polygenic Risk Score for Molecular Vulnerability to Depression Predicts Brain Function During Face Processing

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**Background:** Major depressive disorder (MDD) is the leading cause of years lost due to disability worldwide. Still, the pathophysiological mechanisms of this common and debilitating disease are poorly understood. Imaging and postmortem gene expression studies converge to associate MDD with dysregulation of a distributed corticolimbic circuitry (CLC), within which the amygdala serves as a circuit hub. We aimed to determine whether SNPs causing subtle shifts towards a more depression-like transcriptome in postmortem CLC brain tissue might impact brain function and related depression risk in young healthy adults.

**Methods:** Functional magnetic resonance imaging (fMRI), genetics and mood were assessed in 482 non-Hispanic Caucasian young adults (226 men; mean age 19.78 + - 1.23) participating in the Duke Neurogenetics Study (DNS). A transcriptome-based polygenic risk score (T-PRS) characterizing depression-related gene expression changes in the CLC was developed based on a gene list generated by a prior meta-analysis of case-control postmortem brain transcriptome datasets ($n = 101$ postmortem subjects; 50 MDD, 51 controls). This meta-analysis identified 566 genes with consistent changes in gene expression in MDD, which were associated with altered neurotrophic support, brain plasticity and neuronal signaling (Ding et al, 2015). Next, we used the PrediXcan tool (Gamazon et al., 2015) to "impute" cortical expression levels of 102 out of these 566 genes into the DNS, using the GTEx cortex tissue as a reference transcriptome. A polygenic risk score was created as a sum of these imputed expression values, each weighted by previously reported effect size and direction of association with depression, such that higher T-PRS reflects a more depression-like CLC transcriptome. Mood was assessed using the Mood and Anxiety Symptom Questionnaire (MASQ), which provides information on shared (e.g. general distress) as well as distinct (e.g. anxious arousal, anhedonia) aspects of anxiety and depression. fMRI data was acquired during the "Hariri Hammer" face-matching task (presenting angry, fearful or neutral faces, and shapes as a control condition),and processed in SPM8.

Statistical analyses: First, hypothesis-driven analyses focusing on amygdala reactivity were conducted using a MANOVA and posthoc linear regressions to evaluate the relationships between T-PRS, sex and amygdala response during three pre-selected contrasts of interest (Emotional faces vs. Shapes, Emotional faces vs. Neutral faces, Neutral faces vs. Shapes). Additional exploratory analyses used partial least squares (PLS) regression to identify latent variables (LVs) capturing shared variance between T-PRS, task condition, and whole-brain response. As a control analysis we also examined the relationship between the novel T-PRS and an MDD PRS based on the latest genome-wide association study of the Psychiatric Genetics Consortium MDD working group (PGC-PRS; Wray et al., 2018).Self-reported sex was modeled as a moderator in all analyses.

**Results:** The MANOVA showed a three-way interaction between T-PRS, sex, and task contrast on amygdala response ($F(5,2390) = 4.05$, $p = 0.001$). Posthoc analyses revealed an interaction between the T-PRS and sex during the Neutral faces vs Shapes contrast ($beta = -0.10$, $p = 0.02$), but not any contrasts containing threatening faces. Higher T-PRS predicted lower response in left amygdala in men ($beta = -0.14$, $p = 0.04$, $R2 = 0.02$), but not women ($beta = 0.06$, $p = 0.36$). Further, lower amygdala response was associated with greater self-reported anhedonia (left amygdala: $beta = -0.09$, $p = 0.04$, $R2 = 0.01$) and these effects were independent of sex as well as the other three MASQ domains. A moderated mediation analysis revealed that in men, left amygdala function mediated the relationship between T-PRS and increased anhedonia ($ab = 0.01$, $SE = 0.01$, 95% CI [0.0007, 0.0364]). The whole brain PLS analyses identified one significant LV, explaining 38.8% of the variance shared between T-PRS, task condition and whole-brain activity. The LV was associated with a distributed network of regions, including key CLC nodes such as the amygdala, anterior cingulate and mid-frontal cortices, as well as clusters spanning parts of the hippocampus, insula, dorsal

striatum and cerebellum. With increasing T-PRS, these regions showed blunted response to neutral faces in men and higher response to shapes in women. While the PGC-PRS did not correlate with our T-PRS ($r = 0.05$, $p = 0.31$), higher PGC-PRS was also associated with lower response to neutral faces in the left amygdala (beta = -0.12, $p = 0.01$, $R2 = 0.01$) and this effect was independent of sex. When included in the same model, both PRS retained their independent effects (PGC-PRS: beta = -0.12, $p = 0.007$; T-PRS*Sex: beta = -0.11, $p = 0.02$) and collectively accounted for more amygdala reactivity variance ($R2 = 0.03$) than each individually.

**Conclusions:** We demonstrate that genetically driven shifts towards a depression-like transcriptome in the CLC may be associated with a male-specific anhedonic pathway of depression risk characterized by blunted reactivity to social stimuli. Our results also suggest that the T-PRS reflects different biological pathways than the PGC-PRS and that greater proportion of variance in amygdala reactivity might be explained when modeling both of these PRS together. Finally, our PLS results suggest the novel T-PRS may be associated with dysregulation in a broader network, including, but not limited to the CLC.

**Keywords:** Depression, Polygenic Risk Score, Amygdala, Functional Magnetic Resonance Imaging, Transcriptome

**Disclosure:** Nothing to disclose.

### T136. Resting State Quantitative Electroencephalography (qEEG) Demonstrates Differential Connectivity in Adolescents With Major Depressive Disorder

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**Background:** Early recognition, diagnosis and prognosis determination in youth with Major Depressive Disorder (MDD) is challenging. Quantitative electroencephalography (qEEG) has emerged as a potential intermediate biomarker in adults, but no data are available for MDD youth. Adults with MDD show an overall increase in resting oscillatory synchrony (OS), a measurement of the brain's connectivity and adjustment capacity, when compared with healthy controls (HC). In particular, the frontopolar midline electrode region has increased OS in the 2.5-12 Hz frequency range in MDD adults. In addition to changes in OS, several adult studies found increased resting alpha wave activity in MDD versus HC in the frontal electrodes. However, some smaller adult studies have seen decreased absolute alpha power in MDD when compared to HC.

This cross-sectional comparative pilot study investigated brain region connectivity using qEEG in MDD youths vs. youth HC. We hypothesized that qEEG can identify differences in EEG spatiotemporal properties in youth with MDD vs HC, similar to that in adults. Specifically, we expected increases in both OS and absolute alpha power in the frontopolar midline region in youth with MDD vs. HC.

**Methods:** Forty youth, age 14-17, were enrolled from an academic department of child psychiatry outpatient clinic. Youth with MDD ($n = 25$) were age- and gender-matched with HC ($n = 15$). MDD inclusion criteria included a confirmed MDD diagnosis and score $\geq 40$ on the Children's Depression Rating Scale. Exclusion criteria included current or past bipolar or psychotic disorder, any past brain surgeries, implanted shunts, meningitis or seizure disorder. HCs exclusion criteria were any lifetime DSM-IV or -5 psychiatric diagnoses or first or second-degree relatives with mood disorder or psychosis. Resting EEGs were recorded with subjects lying quietly, eyes closed. EEGs used a 32-channel enhanced version of the International 10–20 System of Electrode Placement.

The Fast Fourier Transform (FFT) provides a computational approach to frequency domain analysis of time series data. This model was used to compute the power spectrum of the signal time series and then compute power for each of the four frequency bands (delta, theta, alpha, and beta) for each subject.

OS was quantified using a weighted-network model and computed as coherence. The nodes in the model corresponded to pairs of neighboring EEG electrodes, and connectivity for each pair was computed as the coherence between EEG signals. Coherence values were compared in each of the four EEG frequency bands to identify differences in resting functional connectivity between groups. For each frequency, coherence scores range from 0-1, with higher score indicating more coherence or OS.

Average coherence for all regions of the brain was calculated for each of the four frequency bands and compared between MDD youth and HC. Similarly, absolute power for each frequency band was averaged across all brain regions and compared between MDD youth and HC. Because of adult literature regarding the importance of the frontal region of the brain in both coherence and power, the average coherence and absolute power for the electrodes located in the frontal lobe were calculated in addition to overall averages and compared between MDD and HC.

**Results:** Mean age of youth was 15.77 ($SD = 0.99$), $N = 39$, 76.9% female. Average overall coherence for all brain regions was significantly lower in the theta band among MDD youth ($n = 25$) ($M = 0.60$, $SD = 0.03$) compared to HC ($n = 14$) ($M = 0.62$, $SD = 0.01$); $t(37) = -2.43$, $p = 0.02$.

In the frontal regions, MDD youth had significantly lower coherence ($M = 0.71$, $SD = 0.07$) than HC in the alpha band ($M = 0.76$); $t(37) = -2.1$ $p = 0.04$ and in the theta band (MDD $M = 0.68$, $SD = 0.04$; HC $M = 0.71$, $SD = 0.05$; $t(37) = -2.54$, $p = 0.02$). Multiple individual node pairs also showed significantly lower coherence in MDD vs. HC.

Averaged across all locations, there were no significant differences in power. Average delta power was significantly higher in frontal regions in MDD youth ($M = 3.09$, $SD = 0.76$) vs. HC ($M = 2.36$, $SD = 0.65$); $t(37) = 3.03$, $p < 0.01$. No significant differences in alpha power were seen between MDD and HC in the frontal region. However, multiple electrode pairs significantly differed in power between the groups; MDD youth had higher power in all significant delta and beta waves, and lower power in all significant theta and alpha waves compared to HC.

**Conclusions:** Brain connectivity measured by qEEG differs significantly between MDD youth vs. HC. Overall, youth with MDD show decreased coherence, a measure of OS, in the theta band and no differences in power in any of the four frequency bands when compared with HC. When focusing on frontal regions, MDD youth show decreased OS in the alpha and theta frequency band, and higher power in the delta frequency band. Additionally, individual node pairs differed in coherence and power levels between the groups. This contrasts with adult data showing increased OS in MDD vs. HC. Although inconsistent with our initial hypothesis, findings might be explained by developmental differences in the adolescent vs. adult brain. This pilot suggests the potential of qEEG as an intermediate biomarker for youth with MDD and the need for larger studies to confirm pilot results and investigate the effects of clinical variables such as anxiety and mood symptom type and severity as well as medications on qEEG measures.

**Keywords:** Quantitative Electroencephalography (qEEG), Adolescent Depression, Biomarker

**Disclosure:** Nothing to disclose.

### T137. Symptom Profile Subtypes Predict Treatment Response to 5 Hz Repetitive Transcranial Magnetic Stimulation in Major Depressive Disorder and Co-Morbid Post-Traumatic Stress Disorder

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**Background:** The diagnostic categories defined by DSM often encompass a heterogeneous set of symptom profiles that may reflect differences in the underlying etiology, pathogenesis and prognosis of the condition. Grisanzio and colleagues have demonstrated that some of this heterogeneity can be quantified though dimensional analysis of the Depression Anxiety Stress Scale (DASS) and using this approach, they identified six trans-diagnostic symptom profiles that were associated with unique patterns of neuropsychological performance, neurophysiological measurements (EEG), and measurements of functional capacity (Grisanzio KA, Goldstein-Piekarski AN, Wang MY, Rashed Ahmed AP, Samara Z, Williams LM. Transdiagnostic Symptom Clusters and Associations with Brain, Behavior, and Daily Function in Mood, Anxiety, and Trauma Disorders. JAMA Psychiatry. 2018; 75(2):201-209). The current study investigated whether classifying patients at baseline using these symptom profiles would have prognostic value in predicting treatment response in patients with Major Depressive Disorder (MDD) and comorbid Posttraumatic Stress Disorder (PTSD) receiving repetitive transcranial magnetic stimulation (rTMS).

**Methods:** Clinical rating scale data from 35 subjects with both PTSD and MDD who participated in an open label trial of 5 Hz rTMS were used in the study. Using item level data from baseline DASS scores, three primary components, labeled Anhedonia, Anxious Arousal and Tension, were calculated for each subject based on the principal component analysis described by Grisanzio et al. A linear discriminant model was constructed using a simulation of the Grisanzio et al. data set. This model was used to classify subjects into one of six pre-defined symptom profiles: Normative Mood, Tension, Anxious Arousal, Generalized Anxiety, Anhedonia and Melancholia. Post treatment remission rates and response to rTMS defined by the Inventory of Depressive Symptomatology Self Report (IDS-SR) and percent change in the PTSD Checklist for DSM-5(PCL-5) were then assessed across profile subtypes.

**Results:** All six symptoms profiles were identified in this sample in the following proportions: Anxious Arousal (43%), Anhedonia (20%), Tension (14%), Normative Mood (14%), General Anxiety (6%), and Melancholia (3%). Post-treatment depression remission rates (defined by IDS-SR scores less than 14) differed significantly across symptom profile subtypes (Fisher's exact test (FET), $p = 0.04$), with Anxious Arousal representing the group with the lowest remission rate (13%, $n = 2/15$) and Tension (80%, $n = 4/5$) and Melancholia (100%, $n = 1/1$) representing the groups with the highest remission rates. When compared to all other subtypes, subjects classified as belonging to the Anxious Arousal subtype were less likely to remit compared all other subtypes (FET, odds ratio 0.16, $p = 0.034$). Subjects in the Anxious Arousal subtype also demonstrated nominally smaller reduction on the PCL-5 after rTMS compared to all other subtypes (21% vs. 46%; $t(33) = 2.025$, $p = 0.051$) and were less likely to complete the treatment series (FET, odds ratio 0.066, $p = 0.011$). The Anxious Arousal component score appeared to drive this effect and a simple model using a cutoff score of 1.0 for this standardized component at baseline yielded reasonable separation between subjects who later met post-treatment remission criteria and those who did not, correctly classifying 74% of the sample with sensitivity of 0.59 and specificity of 0.89.

**Conclusions:** This study offers preliminary evidence of the feasibility and utility of applying these trans-diagnostic symptom profiles to other samples using linear discriminant analysis and suggests that these subtypes differ in their response to treatment with rTMS. These results imply that classifying individuals according to these trans-diagnostic symptom profiles may offer a simple and inexpensive method to help guide rTMS treatment decisions.

**Keywords:** Transdiagnostic, Transcranial Magnetic Stimulation, Clinical Predictors

**Disclosure:** Nothing to disclose.

### T138. Sequential Parallel Comparison Design: Impact on Placebo Response in Buprenorphine/Samidorphan Trials of Major Depressive Disorder

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**Background:** High placebo response rates have been identified as a major issue affecting the ability to detect efficacy in clinical studies, leading to challenges in developing new antidepressant therapies. Various study designs have been investigated to minimize the impact of placebo response on trial outcomes in major depressive disorder (MDD). The sequential parallel comparison design (SPCD) strategy was developed to enhance signal detection in the presence of placebo response, as an alternative to the placebo run-in enrichment approach for identifying placebo non-responders (Fava M, et al. Psychother Psychosom 2003;72:115-127). SPCD includes 2 stages: Stage 1, the comparison of drug and placebo in a standard parallel comparison, and Stage 2, the comparison of drug and placebo using an enrichment comparison design with the placebo nonresponders. Estimates of the drug-placebo differences in each stage are averaged to derive the overall primary comparison. Important features of the SPCD are the randomization of patients in both stages, the utilization of all enrolled patients in study analyses (in contrast to exclusion of placebo responders in the traditional placebo run-in study design), and the blinding of placebo allocation in Stage 1, which increases the proportion of patients identified as placebo responders in Stage 1 and lowers the proportion of placebo responders in Stage 2.

**Methods:** Three multicenter, randomized, double-blind, phase 3 trials that investigated the buprenorphine/samidorphan combination (BUP/SAM 2 mg/2 mg, once-daily) in the adjunctive treatment of MDD were analyzed: FORWARD-4 and FORWARD-5 (both of which used SPCD; ClincalTrials.gov ID: NCT02158533 and NCT02218008, respectively) and FORWARD-3 (which used a double-blind, placebo run-in design; NCT02158546). All enrolled patients had a Diagnostic and Statistical Manual of Mental Disorders (DSM) diagnosis of MDD and an inadequate response to 1 or 2 courses of antidepressant therapy during the current episode. Baseline symptom severity and psychiatric history were broadly similar across the 3 trials. Changes in MADRS total score after a 6-week treatment period in Stage 2 (estimated using mixed models for repeated measures) were compared in the BUP/SAM 2 mg/2 mg versus placebo groups in each trial; all patients continued to receive their current antidepressant therapy. Placebo responders were defined as those with a MADRS-10 score < 15 at the end of placebo run-in/Stage 1 or ≥ 50% reduction in MADRS-10 score from baseline to the end of placebo run-in/Stage 1. These

SPCD studies were not powered to detect efficacy using a single stage.

**Results:** In FORWARD-3, the 4-week double-blind, placebo run-in strategy identified 21% of enrolled patients as placebo responders. In contrast, in FORWARD-4 and FORWARD-5, the 5-week Stage 1 of SPCD identified 33% and 27% of patients as placebo responders, respectively. MADRS total score changes after the 6-week treatment period were similar for BUP/SAM across the 3 studies (-4.8, -5.2, and -3.6 in FORWARD-3, FORWARD-4, and FORWARD-5, respectively). In contrast, the placebo MADRS change after 6-weeks was lower for the SPCD studies (-2.1 and -1.9 in FORWARD-4 and FORWARD-5, respectively) than for the placebo run-in study (-4.6 in FORWARD-3).

**Conclusions:** This is the first analysis to compare SPCD versus a traditional placebo run-in approach in contemporaneously-run MDD studies and using the same investigational drug. Across the 3 phase 3 trials that investigated BUP/SAM 2 mg/2 mg as an adjunctive treatment, consistent changes were observed in MADRS-10 total scores after 6 weeks in the BUP/SAM 2 mg/2 mg groups. The main difference in efficacy results between the trials was the size of the placebo response in Stage 2 randomization, which was lower in the FORWARD-4 and FORWARD-5 trials that used SPCD. The SPCD studies were more successful at excluding placebo responders from the second randomization. Reducing the background placebo response is concluded to enhance the detection of signal response. Based on these phase 3 trials that investigated BUP/SAM 2 mg/2 mg, SPCD may be an important study design for improving the testing and development of future MDD therapies.

**Keywords:** Buprenorphine/Samidorphan, Major Depressive Disorder, Sequential Parallel Clinical Design

**Disclosure:** http://mghcme.org/faculty/faculty-detail/maurizio_fava, Advisory Board, Consultant, Patent

### T139. Early Life Stress is Associated With Increased Odds of Cytomegalovirus Infection in Individuals With Major Depressive Disorder

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**Background:** Early life stress (ELS) is a well-established risk factor for the development of mood disorders and medical illnesses in adulthood. The mechanistic pathways underlying this relationship are unclear although immune dysregulation is one possibility. Herpesviruses are potential immune-modifying agents that may persist in the body and brain and can be reactivated by physical or psychological stress. One possibility is that ELS becomes "biologically embedded" by impairing adaptive immunity, thus increasing both vulnerability to initial infection and subsequent reactivation of herpesviruses. Given its prevalence (□50% in the USA) and documented pathogenic effects, we focused on cytomegalovirus (CMV) as a proof-of-principle test of the relationship between ELS and infectious disease in the context of depression.

**Methods:** The current study examined the relationship between ELS and CMV infection in a discovery sample of volunteers who received a DSM-IV diagnosis of major depressive disorder (MDD; n = 179; 46% CMV-positive; age = 36 ± 12; 77% female; 50% remitted) based on the SCID and an interview with a psychiatrist, and a replication sample of volunteers who met DSM-V criteria for MDD (n = 295; 57% CMV-positive; age = 35 ± 11; 62% female; 28% remitted) based on the MINI. Serum IgG antibodies to CMV were quantified by enzyme linked immunosorbent assay. Self-reported ELS was measured with the Childhood Trauma Questionnaire (CTQ). Exclusion criteria for the discovery sample included major medical disorders, substance abuse within the previous six months or lifetime history of substance dependence, and acute/chronic illnesses (or treatments) that would likely impact immunity. The replication sample was drawn from community-based study with fewer exclusion criteria.

**Results:** Logistic regression models controlling for age, sex, and ethnicity revealed that for every point increase on the CTQ (total score, with a possible range of 25-125 points) the odds of testing seropositive for CMV was increased by a factor of 1.02 ($p = 0.043$) in the discovery sample and a factor of 1.02 ($p = 0.005$) in the replication sample. Analysis of the CTQ subscales showed that in both samples, participants with higher self-reported physical abuse (discovery sample - adjusted OR = 1.10, $p = 0.024$; replication sample - adjusted OR = 1.08, $p = 0.018$) and sexual abuse (discovery sample - adjusted OR = 1.07, $p = 0.009$; replication sample - adjusted OR = 1.08, $p = 0.003$) were more likely to test positive for CMV. The results remained significant in both samples when controlling for medication status but were only significant in the replication sample when mood status (depressed versus remitted) was added to the model. IgG titers, an indirect marker of current viral reactivation, were not significantly associated with CTQ scores.

**Conclusions:** Despite substantially different inclusion/exclusion criteria, the strength of the association between ELS and CMV serostatus was consistent across the two samples, suggesting a robust relationship that is not likely to be attributable to comorbidity.

Initially believed to be benign except in cases of immunosuppression, positive CMV serology is now linked with a range of negative outcomes including depression, immunosenescence, neurodegenerative disorders, an increased risk of complications and/or mortality in critically-ill patients, and reduced life-span in healthy septuagenarians. Vaccines are currently under development. Conceivably, chronic reactivation of CMV may be one mechanism through which ELS exerts its pernicious effects on physical and mental health. If substantiated, this finding has important clinical implications given that CMV can be controlled with well-tolerated anti-viral agents that could theoretically be administered prophylactically or during acute depressive episodes.

Besides the caveat that this study is cross-sectional, one limitation is the absence of childhood socioeconomic status (SES) data. Low childhood SES has been associated with CMV infection but importantly this relationship is not attributable to increased exposure to the virus or to adult stress levels. In fact, because ELS is strongly associated with low SES, the previously reported association between CMV and SES is consistent with the current findings. In sum, our study adds vulnerability to CMV (and potentially other latent viruses) to the list of adverse consequences of ELS. It also highlights an avenue of future research that could potentially validate the proposed mechanistic link between herpesviruses and the sequalae of ELS and leverage this potential relationship to improve clinical outcome.

**Keywords:** Major Depressive Disorder, Early Life Stress, Immune Dysfunction, Cytomegalovirus, Herpesvirus

**Disclosure:** Nothing to disclose.

### T140. Suicide Behavior in Adolescents and Young Adults is Associated With Dysfunctional Th2-Cell Mediated Adaptive Immune Response

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**Background:** Aberrant immune response is implicated in the pathophysiology of suicide behavior. As suicide is one of the leading causes of mortality in adolescents and young adults, we evaluated potential immune profile differences between two distinct groups in this age range: those at risk of major depressive disorder but asymptomatic (MDD; at-risk) and those with recent suicide behavior/severe ideation (suicide behavior).

**Methods:** Plasma samples from at-risk (n = 72) and suicide behavior (n = 38) participants (ages 10-25 years) were assayed for chemokines and T helper (Th)1- [Interferon gamma and interleukin (IL) 2], Th2- (IL-4 and IL-10), and non-T- (IL-1β, IL-6, tumor necrosis factor alpha) cell-related cytokines using a Bio-Rad multiplex panel (Bio-Plex Pro Human Chemokine 40-plex). Cytokine and chemokine levels (after log transformation of variables with skewed distribution) were compared between the two groups using linear regression analyses after controlling for age, gender, body mass index, race and ethnicity.

**Results:** Only levels of IL-4 differed significantly (adjusted p = 0.0026) between the groups after false discovery rate (FDR) correction. Participants with suicide behavior had lower IL-4 levels (median = 16.7 ng/ml, IQR = 7.9) than those at-risk (median = 27.7 ng/ml, IQR = 18.4). Levels of IL-10 were also lower in those with suicide behavior (median = 18.1 ng/ml, IQR = 8.9) than those at risk (median = 23.3 ng/ml, IQR = 8.2) before FDR correction (unadjusted p = 0.049, adjusted p = 0.39). There was no significant association between self-reported depression severity and either IL-4 (p = 0.45) or IL-10 (p = 0.46) levels.

**Conclusions:** Adolescent and young adult patients with recent suicide behavior exhibit lower Th2-cell related cytokines, suggesting an autoimmune process. Targeting inflammation presents a promising avenue to reduce suicide behavior.

**Keywords:** Suicidality, Peripheral Biomarker, Inflammation, Depression

**Disclosure:** AcademyHealth, Alkermes Inc., Akili Interactive, Allergan Pharmaceuticals, ACADIA Pharmaceuticals Inc., American Society of Clinical Psychopharmacology, Brain Institute Canada (CAN-BIND), Brintellix Global, Global Medical Education, Healthcare Global Village, Health Research Associates, Jazz Pharmaceuticals, Lundbeck Research USA, Medscape LLC, MSI Methylation Sciences Inc., Nestle Health Science – Pamlab Inc., Naurex Inc., Navitor, One Carbon Therapeutics, Otsuka America Pharmaceutical Inc., Saatchi, Takeda Global Research, Consultant, Janssen Research & Development LLC, Oxford University Press, Royalties, Dartmouth College, Global Medical Education, Univeristy of Illinois Chicago, Univeristy of Ottawa, University of Texas Health Science Center at San Antonio, Honoraria

### T141. Sustained Remission With Adjunctive Brexpiprazole in Major Depressive Disorder: Post Hoc Analysis of a Long-Term, Open-Label, Extension Study

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**Background:** Brexpiprazole is a serotonin–dopamine activity modulator that acts as a partial agonist at serotonin 5-HT1A and dopamine D2 receptors, and as an antagonist at serotonin 5-HT2A and noradrenaline α1B/2 C receptors, all with subnanomolar potency. The efficacy and safety of brexpiprazole as adjunctive therapy to antidepressant treatment (ADT) have been demonstrated in four short-term studies and one long-term extension study in adults with major depressive disorder (MDD) and inadequate response to ADTs. Treatment guidelines emphasize that remission is the ultimate goal of acute treatment in MDD, meaning that the patient should be asymptomatic over an extended period (sustained remission), with minimal disruption to psychosocial and occupational functioning (full functional remission). The aim of this analysis was to calculate the incidence of sustained remission among patients with MDD receiving adjunctive brexpiprazole, based on data from the long-term extension study.

**Methods:** The long-term study (Orion, NCT01360866) was a 52-week (amended to 26 weeks) open-label, uncontrolled study, in which patients received ADT + brexpiprazole 0.5–3 mg/day (flexible dose). Patients could enter Orion if they completed one of three short-term randomized, double-blind, placebo-controlled studies. The lead-in studies enrolled adults with MDD (DSM-IV-TR criteria) and inadequate response to 1–3 prior ADTs. In the lead-in studies, patients received a prospective ADT + placebo for 8–10 weeks; those patients who responded continued on ADT + placebo whereas those patients who showed inadequate response were randomized to 6 weeks of ADT + brexpiprazole, ADT + quetiapine extended-release (XR) (one study only), or continued ADT + placebo. The percentage of patients who achieved remission at Weeks 26 and 52 of Orion was calculated post hoc for all treated patients with post-baseline efficacy data. Sustained remission was defined as a Clinical Global Impressions – Severity of illness score of ≤ 2 for ≥ 8 consecutive weeks.

**Results:** A total of 2,916 patients were evaluated. Considering these patients' prior treatment in the lead-in studies, 1,630 responded to and remained on ADT + placebo, whereas 698 were randomized to ADT + brexpiprazole, 512 were randomized to ADT + placebo, and 76 were randomized to ADT + quetiapine XR. In Orion, the accumulated sustained remission rate was 49.0% at Week 26 and 55.6% at Week 52. For patients who completed 52 weeks of treatment (n = 770) the accumulated sustained remission rate was 67.3%.

**Conclusions:** The goals of treatment in MDD are to achieve sustained remission and to restore functioning. In this analysis, a high proportion of patients achieved sustained remission after 6 months and 1 year of adjunctive brexpiprazole treatment, suggesting that adjunctive brexpiprazole is a valuable long-term treatment option.

**Keywords:** Sustained Remission, Adjunctive Brexpiprazole, Long-Term Treatment of MDD

**Disclosure:** Otsuka Pharmaceutical Development & Commercialization Inc., Employee

### T142. Neurocomputational Mechanisms of Antidepressant Placebo Effects

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**Background:** Antidepressant placebo effects are traditionally thought to depend on prior beliefs about a treatment's effectiveness. Little is known, however, about the mechanisms underlying the evolution of such beliefs and their impact on mood. Reinforcement learning (RL) models provide a formal account of how beliefs are updated. To examine computational mechanisms of antidepressant placebo effects, we applied RL to data from an antidepressant placebo experiment where both placebo-associated expectancies and its ongoing brain effects were independently manipulated.

**Methods:** Thirty depressed patients received 128 intravenous infusions, including two different placebos (a "fast-acting" or "conventional") and two corresponding no-infusion control

conditions. Each infusion or no-infusion condition was followed by independently manipulated sham neurofeedback purporting to reveal the brain effects of the drug (sham brain-signal readouts displayed on a monitor). Participants rated their expected and actual change in mood after each infusion and neurofeedback period, respectively. Our RL Q-learning model predicting evolving expectancies associated with each condition based on initial expectancies and ongoing "neurofeedback" was implemented using the variational Bayes approach. Expectancies for each trial were then examined as predictors of mood improvement in a mixed-effects logistic regression model.

**Results:** Compared to the null model with fixed expectancies, the RL model successfully predicted patient's evolving expectancies of improvement (Bayesian Omnibus Risk < 0.05), suggesting that they are updated based on ongoing experience (as manipulated with neurofeedback). However, we observed a marked heterogeneity in the extent to which individuals' expectancies conformed to model predictions. After controlling for expectancy ratings (Estimate = 1.2, S.E. = 0.1; $p < 0.001$) and neurofeedback (Estimate = 3.1, S.E. = 0.1; $p < 0.001$), RL model-estimated expectancies (Estimate = -0.5, S.E. = 0.6; $p = 0.009$) predicted subjective mood improvement.

**Conclusions:** Expectancies of mood improvement induced by antidepressant placebo evolve in line with predictions of RL, impacting mood. These dynamics are subject to marked individual differences, potentially mirroring individual susceptibility to placebo effects. These results provide a computational framework for biological studies of antidepressant placebo effects.

**Keywords:** Reinforcement Learning, Depression, Placebo Response

**Disclosure:** Nothing to disclose.

### T143. The Emotional Quality Experienced During a Ketamine-Induced Psychedelic 'Trip' Predicts Subsequent Antidepressant Efficacy

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**Background:** Parenteral administration of sub-anesthetic dose ketamine (0.25 -1.0 mg/kg) produces a transient psychedelic experience that is similar to that produced by 5-HT2A agonists such as psilocybin and LSD. This is often followed by a rapid and robust antidepressant effect that typically lasts days to weeks. There is controversy whether the psychedelic experience induced by ketamine is associated with its antidepressant efficacy. This retrospective analysis compared reduction in depression symptoms produced by a single low dose intramuscular (IM) injection of ketamine (0.25 mg/kg) administered in a clinical setting to patients with treatment resistant depression and their ratings in the Psychedelic Experience Survey (PES), a survey developed by the lead author to clinically assess the quality of patients' ketamine-induced psychedelic trip. The PES has 7 items: intensity, dissociation, hallucinations, insight, positive content, negative content and nausea. Patients rate each of these items from 1 (absent) to 5 (very strong) shortly after their psychedelic experience has dissipated. The PES also prompts patients to write a brief narrative description of their trip.

**Methods:** A Pearson correlation was conducted on the change in PHQ-9 scores from pre-treatment with 0.25 mg/kg ketamine to 24-h after the treatment (calculated as a percentage change) and the ratings for each of the items in the PES ($N = 61$).

**Results:** Percentage reduction in PHQ-9 score was most strongly correlated with the positive content ratings ($p = 0.002$). Other ratings that were significantly correlated with percentage change in the PHQ-9 score were: intensity of trip ($p = 0.028$), dissociation ($p = 0.037$), and nausea ($p = 0.037$). Items not significantly correlated with PHQ-9 change were negative content score ($p = 0.223$), hallucinations ($p = 0.165$) and insight ($p = 0.123$),

**Conclusions:** These results suggest that emotional features of the ketamine-induced psychedelic experience, specifically the degree to which its content is experienced as positive, may be the feature that is most strongly predictive of the subsequent antidepressant efficacy of a single treatment with low dose ketamine.

**Keywords:** Ketamine, Depression, Psychedelic Medicine

**Disclosure:** Nothing to disclose.

### T144. Following Ketamine Infusion in Individuals With Treatment Resistant Depression

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**Background:** Ketamine has become a viable treatment for individuals with treatment-resistant major depressive disorder (MDD) (Zarate et. al, 2006). The mechanisms underlying the antidepressant effect of ketamine remain elusive. Reduced blood flow in the basal ganglia has been implicated in depression. (Lafer et. al, 1997). Ketamine, a glutamate receptor agonist, may affect the basal ganglia functioning through NMDA receptors located in the basal ganglia, in particular the caudate, putamen, and nucleus accumbens. Previous studies have linked increased neural activation in the caudate with anhedonia and altered regional cerebral metabolic rate (rCMRGLu) in the putamen (Murrough et al, 2015 & Lally et al, 2014). In addition, alterations in dorsal anterolateral prefrontal cortex functioning have been linked a decrease in glucose metabolism in depression. The purpose of this study is to to examine the relationship between ketamine infusion and changes in neural activation in the caudate, putamen, and nucleus accumbens as well as the dorsal prefrontal cortex using functional Magnetic Resonance Imaging (fMRI).

**Methods:** Study participants were sixteen depressed individuals with treatment resistant major depression stable on their medications for at least twenty-eight days. Patients were treated with Ketamine infusions at the Depression Clinical and Research Program (DCRP) at the Massachusetts General Hospital. For the neuroimaging part of the study, participants completed depression severity assessments (modified Hamilton Depression Scale) and MRI scanning before and after ketamine infusion. Specifically, following the initial MRI scan participants received an open-label infusion of subanesthetic intravenous ketamine (0.5 mg/kg over 40 minutes). Four hours after the ketamine infusion underwent a second MRI resting state scan. Resting state data are currently analyzed using Freesurfer software.

**Results:** Following spatial preprocessing neural activation in the basal ganglia and prefrontal cortex before and after ketamine infusion will be compared using paired t-tests. Preliminary functional analysis results did not reveal any significant changes in functional connectivity between the left caudate and the left putamen, however, this may change once all participants data are analyzed. Additionally, we will report changes in functional connectivity with dorso anterolateral prefrontal cortex and will investigate changes in depression severity before and after ketamine infusion.

**Conclusions:** The results of the study will advance our current knowledge and understanding of the antidepressant effects of ketamine. Specifically, whether ketamine induced changes in acute levels of depression are mediated via changes in neural activation in the basal ganglia and prefrontal cortex.

**Keywords:** Ketamine, MDD, Treatment Resistant

**Disclosure:** Nothing to disclose.

### T145. The National Pregnancy Registry for Psychiatric Medications: Effects of Fetal Exposure to Atypical Antipsychotics on Risk for Major Malformations

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**Background:** Despite widespread use of atypical antipsychotics in women of childbearing potential, systematically ascertained reproductive safety data for these medicines are sparse. The National Pregnancy Registry for Atypical Antipsychotics (NPRAA) at Massachusetts General Hospital was established in 2008 to address this knowledge gap (www.womensmentalhealth.org/pregnancyregistry).

**Methods:** Data are prospectively collected from pregnant women, ages 18-45 years, with three phone interviews conducted at the time of enrollment, 7 months gestation, and 3 months postpartum. The exposed group is comprised of women who have taken one or more atypical antipsychotics during pregnancy; the comparison group is comprised of women with psychiatric disorders who have not taken this class of medication during pregnancy. Information regarding the presence of major malformations is abstracted from obstetric, labor and delivery, and pediatric medical records. Identified cases of major malformations are sent to a dysmorphologist, blinded to drug exposure, for adjudication. The Registry's scientific advisory board, consisting of experts in the fields of teratology, pharmacoepidemiology, and psychiatry, governs the release of findings.

**Results:** As of August 1, 2018, enrollment in the Registry was 1341 women: N = 695 in the exposed group; N = 646 controls. To date, N = 838 women have completed the study and were eligible for inclusion in the current analysis. Medical records were obtained in 83.5% of eligible subjects. At the recent spring (April 2018) scientific advisory board meeting, the advisors recommended the release of outcomes data. Therefore, updated relative and absolute risks of major malformations in the exposed vs. control group will be presented December 2018. At the time of previous analysis in April 2018, the absolute risk of major malformations was 2.7% among infants exposed to an atypical antipsychotic during the first trimester and 0.8% among unexposed infants (n = 662 exposed, n = 600 unexposed; n = 814 eligible for inclusion in analysis). The risk ratio for major malformations was 3.22 (95% CI: 0.92-11.13) comparing exposed to unexposed infants, not reaching statistical significance.

**Conclusions:** The NPRAA is a systematic prospective pharmacovigilance program used to collect reproductive safety information in order to inform the care of reproductive aged women with psychiatric disorders. Forthcoming reproductive safety data will provide updated information from the NPRAA. The importance of pregnancy registries is underscored by recent FDA guidance on drug labeling and the inclusion of the National Pregnancy Registry for Psychiatric Medications in the FDA label for atypical antipsychotics.

**Keywords:** Pregnancy, Women's Mental Health, Atypical Antipsychotics, Congenital Malformations, Psychiatric Disorders

**Disclosure:** Alkermes Biopharmaceuticals, Inc., Consultant, Praxis Pharmaceutical, Consultant, National Institutes of Health, Grant, National Institute on Aging, Grant, Brain and Behavior Research Foundation, Grant

### T146. The NMDAR Antagonist Dextromethadone Increases Plasma BDNF Levels in Healthy Volunteers Undergoing a 14-Day In-Patient Phase 1 Study

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**Background:** Brain-derived neurotrophic factor (BDNF), a neurotrophin widely expressed in the central nervous system, plays an important function in neuronal plasticity. Recently BDNF has been investigated as a biomarker of treatment response in depression and has been implicated in the mechanism of action of ketamine, an N-methyl-D-aspartic acid receptor (NMDAR) antagonist with rapid anti-depressant effects in humans. In a study of patients with treatment resistant depression (TRD), ketamine was found to significantly increase plasma BDNF levels in responders compared to non-responders 4 h post-infusion (Haile CN et al, 2014). Dextromethadone, the d-isomer of the dl-methadone racemic mixture that is used for the treatment of pain and addiction, is undergoing investigation for the treatment of depression. In contrast with the racemic mixture, dextromethadone is free from clinically relevant opioid activity at doses expected to exert NMDAR antagonistic activity in humans; in particular dextromethadone is thought to exert low affinity blocking action on pathologically open NMDA channels. Preclinical studies on animal models standardized for testing response to investigational antidepressant drugs have shown that the activity of dextromethadone is comparable to ketamine in all tested models.

**Methods:** The present study was conducted as part of a single-site, randomized, double-blind, placebo-controlled phase 1 clinical trial of dextromethadone administered orally for 10 days to healthy volunteers admitted for 14 days to a Clinical Research Unit (CRU). Sampling for testing of BDNF plasma levels from one study cohort was performed before any treatment and 4 h after administration of dextromethadone 25 mg (six patients) or placebo (two patients) on days 2, 6 and 10. Plasma levels of BDNF were measured by means of an ELISA kit, following the manufacturer's instructions. Quantitative determination of BDNF was carried out by standard calibration curves obtained with human recombinant BDNF at concentrations ranging from 0.066 to 16 ng/ml (n = 7), processed following the same protocol as the plasma samples. As expected, the calibration curves fitted an allosteric sigmoidal equation ($r2 \geq 0.99$). Each concentration is the result of three independent determinations. Data are presented as mean ± SD. The statistical analyses were performed by means of GraphPad Prism 5.0 and SPSS software. The Wilcoxon Signed Ranks test was performed to compare BDNF concentrations before any treatment and 4 h after administration of dextromethadone or placebo on days 2, 6 and 10. We also checked a Spearman correlation between plasma dextromethadone and BDNF concentrations.

**Results:** In the d-methadone treatment group, 6 of 6 subjects (100%) showed an increase in BDNF levels post dextromethadone treatment compared to BDNF pre-treatment levels (d-methadone group: mean (± SD) = 0.84 ng/ml (0.60); placebo group: mean (± SD) = 0.81 ng/ml (0.38)) with post-treatment day 10 (last day of treatment) BDNF plasma levels ranging from twice to 17 times the pre-treatment BDNF levels (d-methadone group: mean (± SD) = 5.84 ng/ml (2.83); placebo group: mean (± SD) = 0.79 ng/ml (0.30))

($p = 0.028$ at day 2, $p = 0.043$ at day 6, and $p = 0.028$ at day 10, all vs BDNF plasma levels before treatment); the smallest increase on day 10 (twice the pre-treatment level) was seen in the study subject with the smallest day 10 dextromethadone level, Cmax and AUC and the longest Tmax among all six treated subjects, consistent with a lower d-methadone pharmacokinetic disposition with respect to other treated subjects. By contrast, in the two placebo subjects, where as expected dextromethadone levels were 0, the BDNF plasma levels remained unchanged. Plasma BDNF levels measured at day 2 and day 10 were significantly correlated to the plasma levels of dextromethadone when placebo subjects are included in the analysis. At the tested dose of 25 mg per day for ten days dextromethadone did not cause psychotomimetic side effects or clinically relevant opioid side effects. There were no signs or symptoms of withdrawal upon abrupt discontinuation of dextromethadone after the last study drug administration on day 10.

**Conclusions:** The administration of 25 mg of dextromethadone significantly increases BDNF plasma levels in healthy subjects compared to placebo; the increase started at least on day 2 and persisted throughout day 10. Despite the study limitations, its findings add evidence consistent with the results of preclinical studies demonstrating that d-methadone exerts an antidepressant-like activity in animal models of depressed behavior comparable to that of ketamine (in preparation).

Considering the lack of psychotomimetic and opioidergic side effects and the overall acceptable tolerability and safety profile of d-methadone emerged from two Phase 1 studies in healthy volunteers (in preparation), d-methadone has the potential to be a safer and less burdensome treatment alternative to ketamine.

An undergoing Phase 2a study is currently assessing the tolerability, safety and antidepressant efficacy in patients with depression.

**Keywords:** Dextromethadone, NMDA Antagonists, BDNF, Depression

**Disclosure:** Relmada Therapeutics, Employee, Homology Medicines Inc, Stock / Equity, Vertex Pharmaceuticals, Employee (Spouse)

### T147. Rescue of Stress-Induced Suppression of Nest Building Behavior by the Kappa Opioid Receptor Antagonist CERC-501

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**Background:** Stress is associated with increased dynorphin and kappa opioid receptor (KOR) signaling. KOR agonists produce depressive-like effects, including aversion and dysphoria. On the other hand, KOR antagonists exhibit antidepressant-like effects, and are currently under clinical investigation for the treatment of stress-related psychiatric disorders, such as depression. Characterized by persistent negative mood and anhedonia, depression causes reduction of self-care and reduces one's overall quality of life. Nest building behavior may be an ethologically relevant indicator of well-being in mice. Mice ordinarily build nests that may serve for thermoregulation, social attraction, or protection. Impaired nesting produced by stress may be indicative of an anhedonic state. In the current studies, we evaluated the effects of KOR ligands and exposure to stress on nesting. We also investigated sex differences in response to KOR ligands. The following hypotheses were examined: 1) The KOR agonist U50,488 (U50) would suppress nesting; 2) The selective KOR antagonist CERC-501 (formally LY2456302) would block U50-induced suppression of nesting; 3) Females would require higher doses of KOR ligands to affect nesting behavior; 4) Stress exposure would suppress nesting; and 5) CERC-501 would rescue stress-induced suppression of nesting.

**Methods:** In Experiment 1, nesting was assessed in adult male and female C57BL/6 J mice by providing individual mice with compressed square cotton nestlet material. First, injections of U50 (0, 5, or 10 mg/kg, i.p.) ($n = 8$-17 per group) were administered immediately prior to testing. Second, CERC-501 (0, 1, 3, or 10 mg/kg, i.p.) ($n = 10$-21 per group) was administered 24 h prior to vehicle or U50 (10 mg/kg), and nesting was again assessed. In Experiment 2, adult male C57BL/6 J mice ($n = 12$-13 per group) were subjected to four weeks of unpredictable chronic mild stress (UCMS) and nesting was tested weekly during stress and up to 3 weeks after stress recovery. After three weeks of stress, CERC-501 (10 mg/kg) was administered daily for a total of 12 doses. In both experiments, nesting was scored using a scale of 1 to 5 every half hour for up to 5 h. The primary measures included the time it took to reach a criterion score of 3, in addition to the final nest score.

**Results:** In Experiment 1, U50 dose-dependently suppressed nesting at both doses (5 mg/kg: $p < 0.05$; 10 mg/kg: $p < 0.007$) in males. U50-induced suppression of nesting in males was reduced by CERC-501 at 3 mg/kg and 10 mg/kg. In females, nesting was suppressed by U50 only at 10 mg/kg ($p < 0.05$), and the effects of U50 were blocked by CERC-501 only at 10 mg/kg. In Experiment 2, exposure to UCMS reduced nesting and CERC-501 rescued this deficit. Both the effects of stress and CERC-501 remained even after 3 weeks of stress recovery and 15 days of treatment recovery.

**Conclusions:** Nesting is a behavioral measure that is sensitive to alterations by KOR signaling. Activation of KORs by U50 reduced nest building in males, an effect that was blocked by the KOR antagonist CERC-501. The KOR agonist and antagonist affected nesting in females in a similar manner, but higher doses were required of both U50 and CERC-501, indicating that females were less sensitive to the KOR manipulations than males. Overall, these findings agree with earlier preclinical investigations that demonstrated sex-differences in response to KOR ligands. Exposure to chronic environmental stress also produced reductions of nesting behavior in a KOR-dependent manner. The reduction of nesting behavior by UCMS was reversed by treatment with the KOR antagonist CERC-501. Thus, nesting behavior may be a sensitive indicator for the effects of stress and reversal by antidepressants, especially mediated by KOR manipulations. Future studies will address the role of dynorphin secretion, KOR receptor availability, and KOR signaling in mediating these effects in response to KOR ligands.

**Keywords:** Kappa Opioid Receptor, Chronic Mild Stress, Nest-Building, Novel Antidepressant, Sex Differences

**Disclosure:** Nothing to disclose.

### T148. Role of GABAA/AMPA Receptors in Cortical/Raphe Circuit Involved in Ketamine-Induced Sustained Antidepressant-Like Activity

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**Background:** Unlike classic monoaminergic antidepressant drugs, ketamine, an NMDA receptor antagonist, exhibits a rapid and persistent antidepressant (AD) activity, at sub-anesthetic doses in treatment-resistant depressed (TRD) patients and in preclinical studies in rodents. Ketamine unlikely exerts its AD-like activity via NMDA receptor blockade. By stimulating AMPA receptors in Rodents brain, (2 R,6 R)-hydroxynorketamine (HNK), one of its

major active metabolites, would be essential for this activity (Zanos et al. 2016). We recently confirmed that (2 R,6 R)-HNK and ketamine, 24 h post-administration, display an AD-like activity through activation of glutamate, GABA and serotonin neurotransmission in the medial prefrontal cortex (mPFC). Actually, the synaptic excitatory/inhibitory balance was modified by increasing glutamate release by pyramidal neurons and GABA release by interneurons, respectively even though plasma levels of ketamine and (2 R,6 R)-HNK were no longer detected (Pham et al., 2018). These latest results suggest that antidepressant and neurochemical effects of ketamine and (2 R,6 R)-HNK have started a cascade of cellular mechanisms mainly in the mPFC (Pham et al., 2018). Here, we assessed the specific role of various cellular receptor/transporter involved in excitatory - inhibitory neurotransmission such as AMPA and GABAA receptors, glial GLT-1 glutamate transporter in the mPFC-dorsal raphe nucleus (DRN) pathway in mediating the sustained AD-like activity of ketamine, i.e., 24 h post-administration in BALB/cJ mice. We also examined the behavioral and neurochemical consequences of inhibiting the cytochrome P450 enzyme to prevent the metabolism of ketamine to (2 R,6 R)-HNK in the liver.

**Methods:** To further explore the role of mPFC/DRN pathway and also glutamatergic, GABAergic or serotonergic neurotransmissions in ketamine-induced long lasting AD-like activity, different groups of ketamine-treated male BALB/cJ mice were pre-treated with either glutamatergic (NBQX, an AMPA-R antagonist; dihydrokainic acid, DHK, a selective glial glutamate transporter GLT-1 (or EAAT2) inhibitor) or GABAergic (muscimol, GABAA-R agonist) drugs infused either in the mPFC or the DRN. Neurochemical consequences on extracellular levels of glutamate, GABA and 5-HT (Gluext, GABAext, and 5-HText) were measured in the mPFC 24 h after injection using in vivo microdialysis and AD-like activity was determined on the swimming duration in the forced swim test (FST), a commonly used assay that detects antidepressant activity in mice.

**Results:** We found that an intra-DRN NBQX injection blocked the effects of ketamine in the FST and blunted its effects on mPFC 5-HText and Gluext, but not on cortical GABAext. Thus, the sustained ketamine AD-like activity and activation of cortical 5-HT and glutamate neurotransmissions rely on AMPA-R activation in the DRN. Interestingly, a pretreatment with a selective glutamate transporter GLT-1 inhibitor (dihyrokainic acid DHK) mimicked the sustained behavioral and neurochemical effects of ketamine, i.e., increases in swimming duration in the FST and increases in 5-HText, Gluext and GABA ext in the mPFC. As found by Gasull-Camos et al., (2018), it suggests that an increase in excitatory glutamate neurotransmission selectively in the mPFC triggers the sustained AD-like activity of DHK in mice. In addition, an intra-mPFC muscimol injection completely blocked ketamine AD-like activity in the FST and its effects on cortical 5-HText but had no effects on ketamine-induced increase in cortical Gluext and GABAext. It suggests that ketamine AD-like activity and activation of mPFC 5-HT release are limited by GABAA-R activation in the mPFC.

It has been recently suggested that (2 R,6 R)-HNK is not essential for the actions of ketamine mice (Yamaguchi et al., 2018). Here, we found that a pretreatment with fluconazole (10 or 20 mg/kg, i.p.), a CYP3A4 inhibitor, prevented the metabolism of ketamine (10 mg/kg, i.p. given 1 h later) to (2 R,6 R)-HNK and dose-dependently blocked its sustained AD-like activity and mPFC 5-HT release. These data suggest that ketamine metabolites are essential for these responses in BALB/cJ mice, which are known to have an anxiety phenotype.

**Conclusions:** Interestingly, we found that the AD-like activity of ketamine only occurred with a concomitant increase in glutamate and 5-HT release or in the three neurotransmitters glutamate, 5-HT and GABA in the mPFC. However, the cortical excitation-inhibition balance remained constant, highlighting the role of neuronal adaptation in these effects.

Our results question the use of GABAergic benzodiazepines as adjunctive therapy with ketamine in TRD patients with comorbid anxiety. Such information is critical to establish efficacy or treatment restrictions to maximize clinical translation from animal models to TRD patients, effectiveness and safety.

Furthermore, knowing the key role of ketamine metabolites (norketamine and HNKs) in its effects, the present findings highlight the need to pay attention to possible kinetic drug-drug interaction. For example, following co-treatment with ketamine and monoaminergic antidepressant drugs, or with CYP450 inhibitors or activators, that may limit or increase ketamine pharmacological properties, respectively, during its long-term use in the clinic. These preclinical findings may contribute to a better understanding of the cellular mechanisms underlying ketamine AD-like activity.

**Keywords:** Ketamine, (2 R,6 R)-HNK, Glutamate, GABA, Sustained Antidepressant.

**Disclosure:** Nothing to disclose.

## T149. AMPA Receptor Activation-Independent Antidepressant Actions of (S)-Norketamine

Abstract not included.

## T150. Increased Ventral Hippocampal – Prefrontal Coherence After Chronic Social Defeat Stress

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**Background:** Hippocampal and prefrontal cortex volumes are reduced in patients with depression, and preclinical studies have revealed that chronic stress impacts the anatomy and functioning of these structures. Although we have previously demonstrated that ventral hippocampal (vHPC) medial prefrontal cortex (mPFC) communication plays a crucial role in mediating anxiety-related behavior, it remains unknown how chronic stress impacts this communication. Since vHPC-mPFC theta range synchrony is enhanced in negative valence (anxiogenic) environments, we hypothesized that chronic social defeat stress (CSDS) - which leads to social avoidance in susceptible mice - would increase vHPC-mPFC synchrony.

**Methods:** To test this hypothesis, we recorded simultaneous vHPC and mPFC neural activity in adult, male, 129sv mice and compared their synchrony before and after they underwent CSDS ($n = 13$). W used paired non-parametric statistics to compare electrophysiologic measures.

**Results:** We found that CSDS increases multiple measures of vHPC-mPFC theta range synchrony relative to baseline values (coherence $p < 0.05$; power-power correlations $p < 0.05$, phase-phase correlations $p < 0.05$; $n = 13$). Moreover, a sub-analysis revealed that these results held true for susceptible ($n = 9$), but not resilient mice ($n = 4$). This increased synchrony manifested even in ostensibly non-anxiogenic environments, suggesting a persistent circuit change.

**Conclusions:** Collectively, these data suggest that vHPC-mPFC communication plays a role in the manifestation of susceptible behavior seen after chronic social defeat stress.

**Keywords:** Stress, Resilience, Circuit, Synchrony, Susceptibility

**Disclosure:** Nothing to disclose.

### T151. Sex- and Region-Specific Changes in Neural Network Activity in Stress-Susceptible Rats in the Chronic Unpredictable Stress Model of Depression

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**Background:** Major depressive disorder (MDD) is currently the leading cause of disability worldwide and is two times more prevalent in women than in men. The mechanisms associated with the increased female vulnerability to depression is unknown. Aberrant neural oscillatory activity within the putative depression network is an emerging mechanism underlying MDD. However, sex differences in neural oscillatory activity and its contribution to depression susceptibility remains poorly characterized. We therefore sought to evaluate sex differences in innate and stress-induced neural circuit dysfunction within regions of the depression network and whether these oscillatory signatures can predict the subsequent manifestation of depression-like behaviour.

**Methods:** Baseline behaviours in the forced swim test (FST), elevated plus maze (EPM), and sucrose preference test were first collected from male and female Wistar rats, after which rats were stereotactically implanted bilaterally with stainless steel electrodes into the prefrontal cortex (PFC), cingulate cortex (Cg) and nucleus accumbens (NAc). Following recovery, baseline local field potential (LFP) recordings were taken for 30 minutes. All rats were then exposed to mild chronic unpredictable stress (CUS), comprised of various non-debilitating and uncontrollable physical and psychological stressors. To elucidate stress-induced changes in circuit function and behaviour LFP recordings were taken three times a week, with FST and EPM behaviours re-assessed weekly. The CUS procedure was stopped once half of the animals (within each sex) exhibited a depression-like phenotype. Stress-susceptible animals were characterized by a minimum 60% increase in FST immobility whereas animals were labeled stress-resilient if they did not increase in immobility by more than 10% from baseline. Animals were run in two cohorts, with total sample sizes as follows: stress-susceptible females ($N = 9$), stress-resilient females ($N = 10$), stress-susceptible males ($N = 8$), stress-resilient males ($N = 9$). The estrous cycle stage in the females was determined prior to all recordings and behavioural tests with vaginal lavage. Chronux software for MATLAB was used to evaluate the spectral power at each frequency band within each region with the behavioural outcomes and the stage of the estrous cycle.

**Results:** Consistent with the known enhanced female responses to stress, a shorter CUS exposure was sufficient to induce depressive-like behaviour in stress-susceptible females (3 weeks) compared to stress-susceptible males (5 weeks) in both cohorts. Analysis of the LFP data from the second cohort is currently underway. Preliminary data from the first cohort of animals indicate the presence of innate sex- and region-specific differences in the baseline power spectra. However, no differences in the baseline spectral power at any frequency between stress-susceptible and stress-resilient male or female animals are as yet evident ($N = 3$-5/group). Following CUS, stress-susceptible male and female rats displayed within subject changes in the power spectra not exhibited by the resilient rats, and early evidence indicates a possible correlation between these changes and the manifestation of depression-like behaviour. Estrous cycling was disrupted during the last week of CUS exposure for both resilient and susceptible females.

**Conclusions:** Preliminary findings infer that there exist sex differences in stress-induced oscillatory signatures that may accompany the expression of depression-like behaviour. Analysis of the second cohort of animals to increase sample sizes is ongoing. Temporal changes in network function throughout the CUS protocol are also being assessed.

This study was supported by a grant from the Canadian Institute for Health Research

**Keywords:** Depression, Sex Difference, Oscillatory Activity, Chronic Mild Stress

**Disclosure:** Nothing to disclose.

### T152. Anhedonia, Reward Responsivity, and Anterior Insula Functional Connectivity in Unipolar Vs. Bipolar Depression

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**Background:** Unipolar and bipolar depression are clinically indistinguishable during depressed mood. However, the question remains as to whether specific underlying dimensions of depressive episodes might differentiate the disorders. Anhedonia is a key dimension of reward responsivity that is exacerbated during depressive episodes. At the level of neurocircuitry, the anterior insula is a key region implicated in the regulation of reward responsivity through the mapping of reward prediction errors to regulatory responses. Here we examine whether the relationship between anhedonia, reward responsivity and anterior insula functional connectivity to reward, salience and regulatory regions differentiates unipolar from bipolar depression.

**Methods:** 35 unipolar and 24 bipolar depressed adults completed the Snaith-Hamilton Pleasure Scale (SHAPS) and completed a resting-state fMRI scan. Patients performed the Probabilistic Reward Task (PRT), designed to measure modulation of behavior based upon prior positive reinforcements. Differences between groups across self-report, behavior and functional connectivity were analyzed using MANCOVA controlling for age, gender and medication load. Post-hoc pairwise comparisons were conducted with Bonferroni correction. The relationship between self-reported reward, behavioral performance, and functional connectivity were examined using partial correlations controlling for age, gender and medication load.

**Results:** Unipolar depression was distinguished from bipolar depression by increased anhedonia ($Meandiff = -25.33$, $p < .00001$); reduced reward response bias ($Meandiff = -.33$; $p = .05$) and increased reward reaction time ($Meandiff = 10.95$, $p < .0001$). Increased anhedonia was significantly associated with reduced insula-nucleus accumbens functional connectivity ($r = .30$, $p = .05$). Reduced reward response bias was significantly associated with decreased insula-nucleus accumbens functional connectivity ($r = .44$, $p = .006$) and increased insula-inferior parietal lobule functional connectivity ($r = -.37$; $p = .02$). Slower reaction time to reward was significantly associated with decreased insula-amygdala connectivity ($r = -.31$; $p = .04$).

**Conclusions:** Unipolar depression is associated with increased anhedonia and reduced response bias towards rewarding stimuli over time relative to bipolar depression, indicating both impaired reward responsivity and deficits in positive reinforcement learning. Reduced positive response bias was associated with increased insula connectivity to cognitive control regions and decreased insula connectivity to salience and reward processing regions, suggesting that reduced behavioral adaptation to positive reinforcement may be associated with reduced saliency and reward signaling in unipolar depression.

**Keywords:** Mood Disorders, Anhedonia, Reward Functioning, Resting State Functional Connectivity

**Disclosure:** Nothing to disclose.

## T153. Toward Building a Causal Connectome Atlas of the Healthy Brain

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**Background:** This study supplements existing connectome studies focused on large population statistics by collecting a large amount of single pulse Transcranial Magnetic Stimulation and Electroencephalography (spTMS-EEG) data from a single individual. This N-of-1 study utilizes focal TMS perturbations at different cortical targets spanning all TMS accessible scalp locations to produce an atlas of causal brain responses. In addition to spatially sampling of the brain with spTMS-EEG, high-fidelity structural and resting state magnetic resonance imaging (MRI) scans are acquired in this study from the same individual, which allows the TMS-EEG findings to be better integrated with results from other studies such as the human connectome project.

**Methods:** This study was approved by the ethics review board and includes one healthy volunteer (male, 32). TMS was applied with a figure-8 coil at 120% of the resting motor threshold of the left first dorsal interosseous muscle, while EEG was collected with a 96-channel system. TMS stimulation sites were chosen based on two heuristics: 1) cortical regions corresponding to nodes of resting state networks, 2) cortical regions under the recording electrodes based on the individual's scalp anatomy and are a subset of the electrodes part of the 10-5 international system. In total, 116 cortical sites were completed with neuronavigation and in a randomized order. To account for current orientation effects, each site was targeted twice, once with the backward pointing handle 45 degrees clockwise and once with the handle 45 degrees counter clockwise relative to the anterior-posterior axis. Each recording condition had 100 trials and the entire experiment lasted for 18 recording days over the course of two months. Complementary to the intensive spTMS-EEG experimental protocol, the participant also underwent a series of structural and functional MRI scans, which included twelve 10 min resting state functional MRIs (rsfMRI), six 2-shell 150 direction diffusion weighted imaging (DWI) scans, five T1 scans, and one T2 scan.

spTMS-EEG data was preprocessed with the lab's automated artifact rejection algorithm (ARTIST). To construct an accurate forward head model, individual cortical and scalp surfaces were calculated from the individual's T1 scans, while improved segmentation of the skull and estimation of the anisotropic white matter conductivity across the brain were performed using T2 and DWI scans respectively. The head model was used to calculate both the electric field map of the TMS stimulus at each cortical target and the brain source time series corresponding to the EEG output response. The 120 min of rsfMRI was used to create an individual functional parcellation, while the six DWI acquisitions were combined to create a high-fidelity individual tractography.

**Results:** To understand how the spTMS-EEG data relates to existing whole brain network maps, we created a causal parcellation map or atlas based on different features of the TMS-evoked responses, using measures at the single sensor/source level and at the level of regional/global interactions. To create the spTMS-EEG based causal atlas, we developed: 1) a mapping function from TMS target to the observed EEG response, and 2) a clustering approach to group the causal spTMS-EEG responses into informative brain networks. Using the rsfMRI and DWI data, we also created single-subject network maps in order to evaluate the amount of information in the spTMS-EEG causal atlas that can be explained by structural and non-causal functional connectivity measures.

**Conclusions:** This causal connectome mapping study is one the first of its kind and has by far the largest number of TMS-EEG stimulation sites in a healthy individual. A causal connectome atlas of the healthy brain can serve as a reference for designing personalized neuromodulation-based medicine in the near future.

**Keywords:** TMS EEG, human connectome, Connectivity-Based Parcellation, electrical field modeling, Diffusion Weighted Imaging

**Disclosure:** Nothing to disclose.

## T154. A Midbrain Dynorphin Circuit Underlies Stress-Induced Fear Generalization

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**Background:** Generalized fear and anxiety are debilitating disorders whose neural circuit underpinnings remain poorly resolved. We recently demonstrated that dopamine neurons of the ventral tegmental area (VTA) reflect behavioral uncertainty that underlies fear generalization. Based on these findings, we hypothesized that this uncertainty is the result of a stress-induced impairment in associative learning processes. Dynorphin (Dyn) and its cognate receptor kappa opioid receptor (KOR) are broadly implicated in stress, dysphoria, and modulation of the dopamine system. Based on this link, we hypothesized that Dyn/KOR signaling in the VTA underlies stress-induced uncertainty associated with fear generalization. Here, we tested this hypothesis using a variety of viral, genetic, and optogenetic strategies in mice. We identified a population of Dyn-producing neurons in the dorsal raphe nucleus (DRN) that project to the VTA to drive elevated fear-induced uncertainty.

**Methods:** KOR-dependence of fear generalization was established by systemic injection of KOR antagonist norBNI (10 mg/kg) or vehicle in distinct cohorts of mice fear conditioned with increasing foot shock intensity (0.1 – 0.5 mA). VTA-dependence of Dyn inputs was established by inactivation of the pro-dynorphin gene (Pdyn) in all inputs to the VTA by injection of CAV2-Cre into the VTA of Pdynlox/lox mice. VTA-dependence of KOR expression was established by cell-type specific viral-mediated CRISPR/Cas9 mutagenesis of Oprk1 in DAT-Cre mice. The source of Dyn inputs to the VTA were established by retrograde mapping through injection of CAV2-FLEX-ZsGreen into the VTA of Pdyn-Cre mice and validated by anterograde mapping through injection of AAV1-FLEX-Synaptophysin-GFP into the nucleus accumbens, BNST, and DRN. Functional analysis of the top Dyn inputs to the VTA in the regulation of fear generalization were performed by optogenetic stimulation of designated inputs to the VTA using viral mediated expression of channelrhodopsin (ChR2) in the BNST or DRN and optical cannulation of the VTA.

**Results:** We find that fear discrimination follows and inverted "U" shape as threat intensity increase, consistent with stressed-induced impairments in associative learning. This effect was blocked by systemic injection of norBNI. Inactivation of Pdyn from inputs to the VTA prevented fear generalization in mice fear conditioned with high-intensity foot shock. Similarly, cell-type specific mutagenesis of Oprk1 (KOR) also prevents fear generalization. Retrograde and anterograde mapping of Dyn inputs revealed three sources, the DRN, BNST, and nucleus accumbens (in rank order from highest to lowest). Stimulation of DRN-Dyn inputs to the VTA promoted fear generalization in mice fear conditioned with moderate-intensity foot shock that does not promote generalization in control mice. This effect was not observed in mice with BNST-Dyn inputs to the VTA optogenetically stimulated.

**Conclusions:** Our findings demonstrate that Dyn/KOR signaling in the VTA plays an essential role in stress-induced associative

learning deficits as threat intensity increases. We delineate a midbrain circuit from the DRN to the VTA that underlies this stress-induced generalization. The findings have broad implications for the molecular and circuit basis of fear generalization and further link stress and uncertainty to this phenomenon.

**Keywords:** Dynorphin, Kappa Opioid Receptor, Fear Generalization, Dopamine

**Disclosure:** Nothing to disclose.

### T155. Inhibiting Ventral Dentate Gyrus Activity Reduces Anxiety-Like Behavior

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**Background:** The ventral hippocampus is crucially involved in stress responses and emotional behavior. We have previously shown that adult neurogenesis in the ventral dentate gyrus of the hippocampus can confer resilience to chronic social stress in mice. At the microcircuit level, adult-born neurons silence the activity of developmentally-born, mature granule cells in the ventral dentate gyrus during the experience of stress and during anxiety-like behavioral tasks. This inhibition of the ventral dentate gyrus is sufficient to confer resilience to chronic stress, as direct inhibition of ventral dentate gyrus mature granule cells can protect from stress-induced behavioral abnormalities. Here, we wanted to investigate potential molecular targets that could be harnessed by new pharmacological interventions to inhibit dentate gyrus activity and to treat or protect from stress-induced psychopathology. We specifically investigated the serotonin 1 A receptor (5-HT1A) in mature granule cells of the dentate gyrus, which has been shown to be necessary and sufficient for the antidepressant effects of selective serotonin reuptake inhibitors in mice.

**Methods:** We used transgenic male mice with a germline deletion of the 5-HT1A receptor (5-HT1A knockout mice) and 5-HT1A knockout mice in which the expression of the 5-HT1A receptor is rescued under the Nrip2 promoter in mature granule cells of the dentate gyrus (DG1A rescue mice). Mice were exposed to 10 days of social defeat stress and subcutaneously injected with saline or with the 5-HT1A agonist, 8-OH-DPAT, at 3 mg/kg daily 30 min before each physical defeat. Control mice were injected with saline or 8-OH-DPAT for 10 days but not exposed to social defeat. All mice were then tested in a social interaction test and in the open field test to assess social avoidance and anxiety-like behavior, respectively. Immunohistochemistry for the immediate early gene, c-fos, was used to assess dentate gyrus activity. Data were analyzed using 2-Way ANOVA with Fisher's posthoc test. Data are presented as mean ± s.e.m.

**Results:** Following 10 days of social defeat stress, saline-treated DG1A rescue mice spent less time interacting with a novel mouse in the social interaction test compared with saline treated control mice (control: 107 ± 10 sec, $n = 10$; defeat: 51 ± 9 sec, $n = 19$; $p = 0.0007$). Daily 8-OH-DPAT injections to activate 5-HT1A receptors in dentate gyrus granule cells did not change social avoidance behavior in undefeated control mice (91 ± 11 sec, $n = 10$). However, in defeated mice, 8-OH-DPAT significantly counteracted the defeat-induced reduction in social interaction time (by 71%, $n = 18$; $p = 0.009$). In the open field test, DG1A rescue mice exhibited less exploration of the anxiogenic center of the arena compared with saline treated control mice (control: 5.7 ± 0.006% exploration, $n = 10$; defeat: 3.4 ± 0.008%, $n = 19$; $p = 0.02$). 8-OH-DPAT did not change open field center exploration in undefeated control mice (6.5 ± 0.007%, $n = 10$). However, in defeated mice 8-OH-DPAT significantly counteracted the defeat-induced reduction in center exploration time (by 74%, $n = 18$, $p = 0.002$). No effects of 8-OH-DPAT were observed in 5-HT1A knockout mice in either the control or the stress condition. Immunohistochemistry analysis for the immediate early gene, c-fos, after social defeat revealed a reduction in the number of c-fos + cells in the ventral dentate gyrus of 8-OH-DPAT treated mice compared with saline treated mice (saline: 31 ± 4 cells per hemisection, $n = 5$; DPAT: 17 ± 3 cells, $n = 6$; $p = 0.04$).

**Conclusions:** Our findings suggest that activating 5-HT1A receptors specifically in granule cells of the hippocampal dentate gyrus can inhibit the activity of the dentate gyrus and confer resilience to stress-induced social avoidance and anxiety-like behavior. Developing new pharmacological compounds to target 5-HT1A receptors in the dentate gyrus could thus be a promising new strategy to treat or protect from stress-induced psychopathology in humans.

**Keywords:** Serotonin 1a Receptor, Ventral Hippocampus, Anxiety, Mood

**Disclosure:** Nothing to disclose.

### T156. NYX-2925 Facilitates Mismatch Negativity and Auditory-Evoked Long-Term Potentiation in Rats: A Translational Biomarker Approach for Measuring NMDAR-Dependent Synaptic Plasticity

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**Background:** NYX-2925 is a novel, oral, small-molecule NMDA receptor (NMDAR) modulator originating from a spiro-beta-lactam based chemical platform and is distinct from known NMDA receptor agonists or antagonists. NYX-2925 is in development as a therapy for chronic pain and is currently under evaluation in two Phase 2 clinical studies, one in subjects with painful diabetic peripheral neuropathy and the other in subjects with fibromyalgia (ClinicalTrials.gov identifiers: NCT03219320; NCT03249103). NYX-2925 facilitates synaptic plasticity as measured by enhancement of long-term potentiation (LTP) both in vitro and ex vivo 1-7 days post-dosing (1-10 mg/kg, PO). NYX-2925 also enhances NMDAR-dependent positive emotional learning and novel object recognition. Auditory event-related potentials (ERPs) as measured by scalp electroencephalogram (EEG) electrodes represent biomarkers that are well conserved from lab species to humans. Using this approach, both mismatch negativity (MMN) as well as auditory-evoked LTP (aLTP) has been shown to reflect a non-invasive measure of NMDAR-dependent synaptic plasticity.

In this study, NMDAR-dependent MMN, as well as NMDAR-dependent aLTP were used as in vivo measures of the enhancement of NMDAR activity by NYX-2925.

**Methods:** ERPs and resting EEG were recorded from brain surface EEG electrodes in 2-3-month-old male Sprague-Dawley rats. A frequency deviant mismatch negativity protocol was used. aLTP was induced by an auditory tetanus (10 standard tones per second for 5 min) and measured by the evoked response to the standard tone 1-hour post-tetanus. Either NYX-2925 (0.1, 1, 10 mg/kg PO) or vehicle was administered 1 h before mismatch negativity and 1-7 days before aLTP testing.

**Results:** NYX-2925 (0.1 and 1 mg/kg PO) increased NMDAR-dependent MMN in rats. aLTP was enhanced by NYX-2925 (1, 10 mg/kg PO) 1-7 days post-dosing.

**Conclusions:** NYX-2925 enhances NMDAR activity in rats as measured by NMDAR-dependent MMN and aLTP. MMN, which assesses early cognitive processing of novel sensory information

and is theorized to manifest a form of NMDAR-dependent plasticity, was enhanced. These results demonstrate the potential utility of these EEG measures as real-time biomarkers of drug activity.

**Keywords:** NMDA Receptor, Mismatch Negativity, Long Term Potentiation, Experimental Therapeutics

**Disclosure:** Aptinyx Inc., Employee

### T157. An RNA-Seq Transcriptomic and In Situ Hybridization Assessment of Mu and Kappa Opioid Receptors and Pro-Enkephalin and Pro-Dynorphin Transcripts in Nociceptive Pain Circuits

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**Background:** The expression of opioid receptors and peptides at many levels of the neuraxis, coupled with observations that opioids microinjected into multiple brain regions can exert an analgesic effect, is consistent with the idea that the analgesia produced by mu-opioid receptor agonists is the summation of distributed actions at many CNS sites. One site of proven efficacy is the spinal cord. While the ability of opioids administered at this site to control moderate to severe pain is an acknowledged fact, the exact cellular compositions of receptors and ligands in the dorsal horn and potential coexpression matrices have not been fully delineated. These factors were addressed using a combination of in situ hybridization, immunohistochemistry, and RNA-Seq transcriptomics.

**Methods:** The rat dorsal horn transcriptome was examined at baseline and at 2 h and 2 days following an experimental peripheral inflammation using RNA-Seq. The two time points correspond to activation of immediate early genes and up-regulation of potential target genes. Expression of the mu opioid receptor protein also was localized by immunocytochemistry. Neurons expressing the mu opioid receptor gene (Oprm1), the kappa opioid receptor gene (Oprk1) and endogenous opioid peptides deriving from the proenkephalin (Penk) and prodynorphin (Pdyn) genes were identified by in situ hybridization. Multicolor fluorescent hybridization was used to ascertain colocalization. The DRG was also examined since it supplies presynaptic inputs to the second order spinal cord neurons. Endpoints were levels of expression in sFPKM (significant fragments per kilobase per million aligned reads), counts of the number of cells and their area and intensity of fluorescent signal, as well as location since primary afferent inputs are arranged somatotopically. We also used the transcriptome results to examine mu receptor splicing.

**Results:** A striking feature observed using in situ hybridization is the abundance of Oprm1-expressing neurons in the superficial layers of the dorsal horn and are also evident using immunofluorescence for mu receptor protein localization. These neurons are difficult to detect with standard immunohistochemical or receptor binding techniques since the dense amount of terminals obscures the presence of the cell bodies. Beyond the dorsal horn Oprm1-expressing neurons could be observed in most of the spinal lamina from IV to X. Kappa receptors were also present and dispersed in a similar pattern as Oprm1-expressing neurons. Penk expression was also mainly concentrated in the dorsal horn although neurons were seen at multiple spinal cord laminae. Pdyn was more confined to laminae I-II and X. During inflammation there was an upregulation of the two neuropeptide genes and this was most pronounced in the medial dorsal horn, both fluorescence intensity and area were significantly increased. While more sections are needed, the expression of Oprm1 and Oprk1 were also upregulated. Quantitation of expression using RNA-Seq in the whole dorsal quadrant showed that Pdyn expression was significantly elevated, but the broad tissue sampling tended to dilute the effect for the other genes. Examination of the DRG showed numerous mu receptor-positive neurons both in vivo and in primary cultures. Intensive analyses of mu receptor transcript splice variants in DRG showed the vast majority were composed of exons 1, 2, 3 and 4. Extended variants at the 5'end or truncated variants that might yield a 6TM protein were very poorly represented, if at all.

**Conclusions:** Our observation that spinal lamina I-II contains two sources of mu-opioid receptors: (a) neurons intrinsic to lamina I-II, and (b) mu-containing terminals arising from DRG neurons, provides a stimulus for re-interpreting how opioids exert analgesic actions at the spinal level, especially in relationship to presynaptic actions of mu agonists. Additionally, these observations suggest that new investigations into the exact synaptic connectivity of the dorsal horn opioid systems are needed in order to understand analgesia and to potentially identify new routes to non-opioid analgesic mechanisms. We show that, at this level of the CNS, the system is dynamically modulated by sustained nociceptive input. In the experimental peripheral inflammation model, we observe significant, strong up-regulation of Pdyn and Penk in a somatotopic distribution that is consistent with afferent synapses from distal limb projections. The multi-label data provides a new a route to discover effective supplementation of the current "mainly Mu" approach to analgesia. Whether sustained nociception induces gene expression programs in regions higher in the CNS needs to be addressed. Such studies may provide further insight into pain and potentially other neuropsychiatric disorders.

**Keywords:** Analgesia, Opioids, Enkephalin, Dynorphin, Mu-Opioid Receptor

**Disclosure:** Nothing to disclose.

### T158. Sleep Disturbance and Environmental Reactivity as Potential Mechanisms for Comorbidity of Mood and Anxiety Disorders With Migraine

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**Background:** There is compelling evidence for an association between migraine with mood and anxiety disorders, but there has been limited research on their potential mechanisms. Previous work has been limited because of the cross-sectional designs, non-systematic sampling, restricted age composition, and lack of incorporation of well-documented heterogeneity of these disorders. Here we employ data from familial aggregation, clinical phenomenology and mobile technologies to examine the interrelationships between subtypes of migraine, mood and anxiety disorders to examine potential sources of associations and their specificity. We further investigate the core phenomena that could underlie this association including sleep patterns, subjective ratings of pain, stress reactivity and emotional regulation using mobile assessments.

**Methods:** The sample included a total of 347 probands and 631 of their directly interviewed first-degree relatives. Structured diagnostic interviews for headache syndromes and mental disorders were administered by experienced clinicians to assess ICHD-II criteria for headache subtypes and DSM-IV criteria for mental disorders. Ecological Momentary Sampling (EMA) was

assessed four times per day over a two-week period (56 assessments in total) in a subset of 289 participants. We evaluated sleep variables, patterns of mood and anxiety, self-reported pain and reactivity to stressful events. Mixed effects models were used to investigate the familial aggregation and co-aggregation of headache subtypes with subtypes of mood and anxiety disorders, and multi-level models that incorporated granger causality were used to analyze the EMA domains and their directionality.

**Results:** We confirmed the well-established familial aggregation of migraine (OR = 2.12 (1.14-3.18), with some evidence for specificity of migraine with and without aura. Strong associations emerged between the Bipolar II and MDD, and all subtypes of anxiety disorders with migraine in relatives (OR = 3.05(1.52-6.11) and OR = 1.63(1.1-2.41), respectively). However, there was no familial overlap in mood/anxiety disorders and migraine. We did not find that people with migraine had greater emotional variability than those with mood/anxiety disorders. However, sleep patterns and disturbances and environmental reactivity were significantly different from controls in people with migraine and those with mood/anxiety disorders. Whereas sleep patterns did not differ in youth with migraine, environmental reactivity was observed across the developmental life span.

**Conclusions:** Although mood/anxiety disorders and migraine are strongly familial, their association does not appear to be familial. Therefore, mood and anxiety disorders are either a precursor or consequence of migraine, rather than comprising manifestations of common etiologic factors underlying migraine and mood disorders. The results of real time assessments among the core domains of mood disorders and migraine suggest that sleep and stress reactivity may potentially mediate their association. Differences in these associations across sex and development also highlight the importance of a developmental perspective in comorbidity research. These findings have important etiologic and treatment implications.

**Keywords:** Migraine, Sleep Disturbance, Mood and Anxiety Disorders, Mobile Technology

**Disclosure:** Nothing to disclose.

### T159. Effects of Threat-Related Early-Life Stressors on Intrinsic Amygdala Activity: Exploring the Neural Substrates of Neuropsychiatric Symptom Elevations in HIV+ Adults

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**Background:** Neuropsychiatric symptom elevations in HIV + adults have recently been linked to lower levels of amygdala response (i.e., blunted reactivity to negatively valenced stimuli). Notably, this response pattern appears to be driven by high levels of early-life stress (ELS) exposure. It has been hypothesized that these ELS-related reductions in amygdala response might actually reflect underlying ELS-related elevations in baseline levels of intrinsic amygdala activity at rest, which contribute to a ceiling effect on amygdala response; however, this hypothesis has not been examined previously. Accordingly, we assessed whether ELS exposure is associated with elevations in baseline levels of intrinsic amygdala activity at rest in HIV + adults. More precisely, we examined whether exposure to a specific type of adverse childhood experience, threat-related stressors (violence exposures), which are known to have a significant impact on affective functions, is associated with elevations in intrinsic amygdala activity. We further examined whether ELS-related elevations in intrinsic amygdala activity accounts for neuropsychiatric symptom elevations in HIV + adults.

**Methods:** Intrinsic activity within bilateral amygdala was assessed during resting-state fMRI in 44 HIV + and 38 HIV-negative control (HC) adults; both male and female participants were included. Standardized self-report measures assessed adverse childhood experiences associated with threat exposure (e.g., sexual, physical, or emotional abuse; domestic abuse; bullying), as well as neuropsychiatric symptom levels (e.g., depression, anxiety, apathy). We examined the independent and combined effects of HIV + status and threat-related exposures on intrinsic amygdala activity using ANCOVA, controlling for demographic variables on which the groups differed significantly (e.g., current stress levels). We further assessed whether intrinsic amygdala activity accounted for neuropsychiatric symptom elevations (composite score) in HIV + adults using linear regression.

**Results:** We observed a significant main effect of threat exposure ($F = 4.06$, $p = .048$) and a trend-level main effect of HIV status ($F = 3.14$, $p = .081$) on intrinsic amygdala activity. Follow-up analyses indicated that these effects were driven primarily by HIV + adults with threat exposure who exhibited significantly greater amygdala activity than HC adults without threat exposure ($t[47] = 2.10$, $p = .041$), as well as by HC adults with threat exposure who exhibited trend-level elevations relative to HC adults without threat exposure ($t[36] = 1.79$, $p = .082$). Regarding neuropsychiatric symptoms, we observed that the HIV + group exhibited elevated neuropsychiatric symptoms relative to the HC group ($F = 4.70$, $p = .034$); however, neuropsychiatric symptom levels were unrelated to levels of intrinsic amygdala activity in the HIV + sample ($\beta = -.001$, $p = .996$).

**Conclusions:** We report the novel finding that early-life exposures to threat-related stressors have a long-term effect on intrinsic amygdala activity at rest. Yet, we also find that the observed ELS-related elevations in intrinsic amygdala activity do not account for neuropsychiatric symptom elevations in HIV + adults. Accordingly, future studies may wish to place greater emphasis on examining ELS-related differences in amygdala reactivity, rather than ELS-related differences in intrinsic amygdala activity at rest, when investigating the neural substrates of neuropsychiatric symptom elevations in HIV + adults.

**Keywords:** Amygdala, HIV, Neuropsychiatric Symptoms (NPS), Adverse Childhood Experiences (ACE), Early life stress

**Disclosure:** Nothing to disclose.

### T160. Surgical Outcome of Extratemporal Epilepsy Due to Focal Cortical Dysplasia Following Non-Invasive Evaluation and Stepwise Intraoperative Electrocorticography

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**Background:** Focal cortical dysplasia (FCD) is the most frequent neocortical pathology underlying partial refractory epilepsies. Surgical outcome is related to completeness of resection of the visible and adjacent microscopic lesion and of the epileptogenic tissue. Extent of resection is often based upon costly preoperative invasive EEG studies. Alternatives to provide reliable delineation of the epileptogenic zone (EZ) following low-cost non-invasive presurgical evaluation are needed. We report surgical outcome of a large series of patients with extratemporal FCD operated following non-invasive presurgical evaluation, with refinement of localization of the EZ through intraoperative, stepwise electrocorticography (ECoG).

**Methods:** 66 consecutive patients with MRI evidence of extratemporal FCD were operated following non-invasive presurgical evaluation. Ictal or continuous epileptogenic discharges (I/CEDs) on acute intra-operative ECoG were present in 58 (89%). Sequential recordings were then performed and complete resection of the cortical tissue displaying I/CEDs, yet preserving eloquent cortex, was attempted. Resections of the lesion and the cortex displaying I/CEDs were deemed complete or incomplete, according to surgical strategy, post-op MRI and sequential ECoG. Lesions were classified as involving or not truly eloquent (i.e., indispensable: rolandic, language) cortex. Histopathology confirmed FCD in all patients (13 FCD type I; 53 FCD type II). Mean and median post-operative follow up were 6.6 and 4.0 years, respectively (range, 1- 21). Outcome was verified prospectively through routine visits to the clinic and classified according to Engel.

**Results:** Complete resection of the lesion was achieved in 40 patients (61%) and of the tissue harboring I/CEDs in 30 of the 58 (52%) with these discharges. Twenty-one patients (32%) were reoperated. Before reoperation, 36 patients (54%) were seizure-free (Engel class I) and, after a second procedure, 40 patients (61%) were in class I. There was a strong correlation between surgical outcome and completeness of resection of the lesion and of the I/CEDs: 33 of the 40 (82%) with complete resection of the visible lesion and 28 of the 30 (93%) with complete resection of I/CEDs were seizure free, compared to 7/26 (27%) and 7 /28 (25%) of those in whom parts of the lesion or I/CEDs remained in place (both $p < 0.0001$). Twenty-four patients (36%) had lesions impinging upon 'indispensable' cortex, and only 7 (29%) were seizure free, compared to 33 of the 42 (79%) of those in whom lesions were in other neocortical regions ($p < 0.0001$).

**Conclusions:** The combination of non-invasive presurgical evaluation and intraoperative sequential ECoG may lead to complete seizure control in a large percentage of patients with complex extratemporal focal dysplastic lesions. Outcome is more likely related to lesion location and resulting feasibility of complete resection of the lesion and relevant epileptogenic tissue – rather than issues of localization of the epileptogenic zone.

**Keywords:** Epilepsy, Seizures, Cortical Dysplasia, Neurosurgery

**Disclosure:** Nothing to disclose.

### T161. Socio-Communicative Deficits are Modulated by GABA Concentrations but Not GABA(A) Receptor Densities in Adults With Autism Spectrum Disorder

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**Background:** Dysfunction of the GABA neurotransmission system is one of the most accepted pathophysiologic mechanisms for autism spectrum disorder (ASD). New imaging technologies allow for in vivo determination of GABA levels by magnetic resonance spectroscopy (MRS) and determination of GABAA receptor densities by [18 F]flumazenil-positron emission tomography ([18 F]FMZ-PET). The goal of this study is to examine the independent and interactive effects of GABAA receptor densities and GABA levels in the thalamus and left dorsolateral prefrontal cortex (DLPFC), on socio-communicative abilities.

**Methods:** Individuals with ASD and neurotypical controls with IQ greater than 70 and age between 18 and 55 years.

Subjects were scanned on GE SIGNA PET/MR (Waukesha, WI). PET data were acquired in list mode, dynamically reconstructed and corrected for photon attenuation using both scanner-specific 8-channel headcoil correction and a MR-measured head atlas-based attenuation correction maps. A reference tissue model (Ichise model; MRTM0) was used to calculate binding potentials (BPND) with pons as the reference region. BPND is a surrogate for GABAA receptor densities.

During PET data acquisition, structural MR and MRS sequences were acquired. An improved MEGA-SPECIAL sequence was performed on the left DLPFC and bilateral thalami with voxel sizes of ~15cc and TE/TR = 80/2000ms, 15 min acquisition time. The IM-SPECIAL edited spectrum was obtained by subtracting the editing OFF spectrum from the editing ON spectrum. GABA levels were estimated from the integrated 3ppm peak area in the edited spectrum divided by the water peak area.

Neuropsychological assessments included Stanford Binet, 5th edition (SB5), Social Responsiveness Scale (SRS-2), Autism Quotient (AQ), Ritvo Autism Asperger Diagnostic Scale-Revised (RAADS-R), and Berkeley Expressivity Questionnaire (BEQ), Social Phobia and Anxiety Inventory (SPAI).

**Results:** Twenty-nine adults with ASD and 29 healthy volunteers participated in this study. When only male participants were included, GABA/Water in the left DLPFC of ASD participants was found to be 46% higher than TD participants ($p = .002$**); in contrast, GABA/Water in the thalami of ASD males was 13% lower than TD males ($p = .041$*). GABAA receptor density in both thalami and left DLPFC did not reveal group differences. When both male ASD and TD participants were combined together, thalamic GABA/Water was found to correlate negatively with the total ($R = -.437$; $p = .029$*), social relatedness subscale ($R = -.472$; $p = .017$*), and sensory motor subscale of RAADS ($R = -.400$; $p = .047$*); on the contrary, left DLPFC GABA/Water correlated positively with the sensory motor ($R = .520$; $p = .009$**) and circumscribed interest ($R = .447$; $p = .028$*) subscales of RAADS. To explore gender differences, we then performed correlational analyses of ASD males and ASD females separately. Thalamic GABA/Water was found to correlate negatively with AQ in male ASD participants ($R = -.630$; $p = .028$*), but positively with AQ in female ASD participants ($R = .813$; $p = .026$*).

**Conclusions:** These results suggested region-dependent and gender-specific nature of the GABAergic system in high-functioning individuals with ASD.

**Keywords:** GABA, GABA-A Receptors, Autism Spectrum Disorder, 1 H MRS, PET Imaging

**Disclosure:** Nothing to disclose.

### T162. Integration of Epigenomic Information Improves Transcriptome Prediction and Identifies Novel Genes in Large-Scale Gene-Trait Association Study

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**Background:** Novel machine learning approaches can generate models for "imputation" (prediction) of gene expression by using solely genetic data as input. These models can be integrated with GWAS to identify changes at the imputed gene expression with much greater power than examining single nucleotide variants. Here, we developed a method that integrates epigenomic information to better estimate variants' effects on gene expression level.

**Methods:** We generated predictive models of gene expression across 14 datasets by using a weighted elastic net model that integrates epigenome data. We applied these models to 58 GWAS to identify genes that are dysregulated in each trait.

**Results:** Compared to previous methods, our model improves the accuracy of gene expression prediction in independent datasets. Integration of gene expression predictors with GWAS summary results identified novel trait-associated genes. These genes are enriched for: (1) biological pathways that are relevant to the etiopathogenesis of the trait, and (2) genes that have been associated with disease-specific Mendelian syndromes, clinical signs and mouse models.

**Conclusions:** Integrating epigenomic information into prediction of transcriptome can improve the performance of imputation and when applied in GWAS data reveals novel tissue-specific trait-associated genes.

**Keywords:** Machine Learning, Epigenome, Transcriptome, GWAS, Neuropsychiatric Disorders

**Disclosure:** Nothing to disclose.

### T163. Endocannabinoids Control the Neural Substrates of Interval Timing in the Nucleus Accumbens

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**Background:** Cannabinoids disrupt timing by interfering with dedicated brain circuits. The ability to perceive and respond to temporally relevant information in the environment is critical for adaptive survival, and corticostriatal circuits play a central role in timing behavior. Our previous work demonstrated that phasic dopamine release in the nucleus accumbens (NAc) encodes interval timing and that CB1 receptor activation accelerates the perception of time and shifts temporally-engendered patterns of phasic dopamine release.

**Methods:** Using in vivo optogenetics, fast-scan cyclic voltammetry, and multiple single-unit recordings, we examined the causal role of NAc dopamine release in interval timing and explored how endocannabinoid signaling orchestrates timing-mediated NAc network dynamics in male mice.

**Results:** We demonstrate that optogenetic stimulation of ventral tegmental area dopamine neurons during the timing interval interfered with timing mechanisms and accelerated time estimation. Additionally, interval timing was encoded by progressive increases in accumbal gamma frequency power of the local field potential. Augmenting levels of the endocannabinoid 2-AG resulted in a leftward shift in the estimate of interval duration and disrupted interval encoding by attenuating gamma frequency oscillations in a CB1 receptor-dependent manner.

**Conclusions:** These results reveal a significant role for the interplay between dopamine and endocannabinoids in accumbal network dynamics that guide timing behavior and may have important implications for the use of pharmacotherapies targeting the endocannabinoid system and for the recreational use of plant-based and synthetic cannabinoids.

**Keywords:** Endocannabinoids, Nucleus Accumbens, Timing, Dopamine, Gamma Oscillations

**Disclosure:** Nothing to disclose.

### T164. A Manualized Eight-Week CBT Group Teletherapy Program for Chronic Low Back Pain Patients: An Ongoing Pilot Study

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**Background:** Chronic low back pain (CLBP), defined as lasting ≥ 3 months, is a massive burden on patients, families, and the health care system. It is a leading cause of disability, reduced quality of life, and suicide. With an annual prevalence of 15-45%, the social and economic costs are immense. In the US, the latter may exceed $600 billion in 2010 dollars, potentially eclipsing those of coronary artery disease, cancer, and AIDS combined.

Further, long-term opioid analgesic use can have deleterious psychiatric, cognitive, and social consequences. Recent pain clinic-based studies suggest rates of prescription opioid misuse (POM) among long-term opioid users are 40-60%. POM is associated with serious health problems, addiction, and increased overdose deaths.

Fear avoidance is also common in CLBP; patients catastrophize about their pain and avoid potential triggers, creating a maladaptive "vicious cycle" leading to social withdrawal, significantly reduced quality of life, and increased anxiety and depression. Opioids do not help this. To address the gap, cognitive behavioral therapy (CBT) has been adapted to treat CLBP, showing durable clinical efficacy. CBT for CLBP includes strategies for activation, problem-solving, cognitive restructuring, and coping skills training; it can directly reduce the experience of pain (e.g. by distraction or reinterpreting the pain experience) and can challenge negative cognitive distortions accompanying fear avoidance.

Unfortunately, many patients cannot access well-trained CBT practitioners due to mobility issues, childcare needs, or living in a remote and/or under-resourced areas. To satisfy this unmet need, remote delivery of CBT has been studied, such as via telephone or online. Following our group's prior work using a manualized 8-week in-person group CBT program developed by Dr. Jamison--in which participants benefit from interaction with each other as well as with the facilitator(s)--we sought to pilot an adaptation of this approach to allow remote participation via secure video conference.

**Methods:** For this ongoing, prospective, open-label pilot study, we adapted a validated pain CBT paradigm developed by Dr. Jamison. We enrolled cohorts of approximately eight participants who participated in the 8-session CBT paradigm via secure WebEx with an MD or PhD-level facilitator. Participants were 18-75 years old with diagnosed CLBP of > 12 weeks' duration with mean daily intensity ≥ 4/10 on a numeric rating scale at least 50% of the time. Participants could not have current substance use disorder, a lifetime diagnosed chronic psychotic or bipolar mood disorder, current litigation related to pain or injury, back surgery < 6 months ago, or planned back surgery in the next 4 months. Baseline (M0), month 2 (M2, end of 8-session CBT program), and month 4 (M4, end of study) participants self-rated pain interference (Brief Pain Inventory [BPI]), low-back-pain-related disability (Oswestry Disability Inventory [ODI]), pain catastrophizing (PCS), POM risk (Current Opioid Misuse Measure [COMM]), and sleep quality (Insomnia Severity Index [ISI]). Participants also completed a study-specific satisfaction survey.

**Results:** This is an in-progress pilot study. Thus far, we have enrolled 29 individuals (69% female); mean ( ± standard deviation) age is 53.3 ± 14.4 years. Ten are actively engaged in the study protocol and 12 have completed all procedures. Five participants voluntarily withdrew and 2 were lost to follow-up. Mean scores decreased for BPI severity (M0: 6.0 ± 1.7; M2: 5.3 ± 1.8; M4: 4.5 ± 1.7) and interference (M0: 6.3 ± 2.1; M2: 5.2 ± 2.3; M4: 5.0 ± 3.0) subscales, ODI (M0: 46.3 ± 15.8; M2: 45.7 ± 13.6; M4: 42.5 ± 19.4), PCS (M0: 21.2 ± 13.5; M2: 18.3 ± 11.7; M4: 15.0 ± 11.7), COMM (M0: 11.3 ± 9.9; M2: 7.0 ± 6.0: M4: 4.3 ± 2.3), and ISI (M0: 15.0 ± 6.8; M2: 12.9 ± 6.7; M4: 10.3 ± 8.1). COMM scores < 9 are considered a negative screen. Mean M2 and M4 ISI scores no longer met criteria for clinical insomnia. Because the study is ongoing, some variables had small numbers (e.g. N = 6 for M4 ratings). Most participants were highly satisfied with the course (73.8 ± 17.7%).

**Conclusions:** Interim results of a telemedicine pain management course are presented. Consistent decreases were found in pain-related interference, disability, and catastrophizing, as well as POM and ISI measures. These results are preliminary due to the limited number of participants completed thus far. The favorable retention and satisfaction rates suggest that the remote video conference group CBT approach is a potentially useful treatment modality for the CLBP population, warranting continuation of this study and future well-powered prospective RCTs.

**Keywords:** Chronic Pain, Cognitive Behavioral Therapy, Teletherapy, Telemedicine

**Disclosure:** Ad Scientiam, Consultant

## T165. VTA Responses to Reward Altered in Chronic Pain: Effect of Sex

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**Background:** Sex differences in both the experience and prevalence of chronic pain are well documented. While research documenting the interactions between sex and pain has markedly increased in recent years, the neurobiological mechanisms which contribute to sex differences in chronic pain still remain poorly understood. Multiple lines of evidence suggest neuroadaptations within reward networks exist in patients with chronic pain conditions. Whether or not men and women with chronic pain conditions exhibit similar alterations in these networks is not currently known. Here, we utilized functional magnetic resonance imaging (fMRI) to explore how reward processing varies between men and women and persons with and without chronic pain conditions.

**Methods:** 51 participants (35 chronic non-specific back pain patients, 16 healthy control subjects) were recruited. Subjects were scanned using fMRI while they performed the monetary incentive delay task which has been demonstrated to robustly elicit responses to anticipation of reward within the nucleus accumbens (NAc) as well as the ventral tegmental area (VTA). Subject data was subjected to GLM analyses using SPM 12, then coregistered and normalized into MNI space for group level comparisons.

**Results:** Whole brain group level analyses indicated significant activation within the NAc bilaterally (LNAC, $pFWE < 0.001$, RNAC, $pFWE < 0.001$) and VTA ($pFWE < 0.001$) in response to anticipated gains relative to neutral trials. A two-way ANOVA was then used to assess the effects of sex (male vs. female) and chronic pain status (chronic pain vs control) on reward anticipatory activation. Analyses indicated a significant interaction between Sex x Chronic Pain within the VTA ($pFWE = 0.044$). Post hoc analyses revealed that female patients exhibited heightened VTA responses to reward relative to female controls, whereas no significant differences between male patients and male control subjects were observed.

**Conclusions:** These data indicate that women with chronic pain conditions, but not men, exhibit altered VTA responses to the anticipation of monetary reward. These results indicate there may be differences in the ways men and women adapt to chronic pain or point to differences in the antecedents of chronic pain conditions between the sexes.

**Keywords:** Monetary Reward, Sex Differences, Chronic Pain

**Disclosure:** Nothing to disclose.

## T166. The Limits of Polygenic Embryo Selection for Cognitive Ability

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**Background:** Given recent developments in genome-wide association studies (GWAS), researchers have increasingly utilized polygenic scores (PGS) as a tool for genetic prediction of complex traits and diseases. While progress in cognitive genomics had lagged behind studies of other anthropometric traits such as height, very recent large-scale GWAS for general cognitive ability have finally permitted the application of PGS approaches to cognition. This has led to an increasing ethical concern, also documented in the popular media, that cognitive PGS could be used to select embryos generated by in vitro fertilization (IVF) for eugenic purposes. Our study quantifies the limits of such applications, based on both present and projected developments in cognitive genomics.

**Methods:** Using genotype and phenotype data of individuals (parents) and offspring across multiple cohorts, we calculated the average difference between: 1) the maximum predicted phenotypic value (based on PGS) and the mean predicted value (the average of the parental values); and 2) the maximum and minimum predicted values (i.e., twice the value of the max-mean difference). We simulated genotypes of embryos (offspring) under simplified assumptions: 10 testable embryos per IVF cycle and no assortative mating. [Note that these assumptions were deliberately selected in order to simulate optimal conditions, and thereby determine the upper limits of prediction; more realistic assumptions of fewer viable/testable embryos and positive assortative mating would tend to reduce the available variance for selection.] We utilized available PGS from large-scale GWAS for general cognitive ability, which currently have a maximum predictive R^2 of ~4-5%. We also examined PGS for height, which are based on larger GWAS and have considerably greater predictive R^2, in order to estimate the future potential of selection.

**Results:** For general cognitive ability, the average difference in predicted cognitive ability between the embryo with maximal PGS and the mean embryo was ~0.115 standard deviations (SD), or roughly 1.75 IQ points. The average difference between embryos with the maximal and minimal cognitive PGS was ~0.23 SD (3.5 IQ points). For height, the max-mean difference was 1.5 cm (~0.2 SD) and the max-min difference was 3 cm (~0.4 SD). More broadly, theory shows that if the variance explained by the PGS is R^2, then the gain in PGS (max-mean) is proportional to R. Moreover, if we have n embryos, the gain in PGS (max-mean) grows approximately as a function of $\sqrt{(\log(n))}$. Thus, a more realistic scenario of ~5 viable and testable embryos would result in values that are reduced by a factor of 0.84, and a future IVF technology permitting 100 viable and testable embryos would increase utility only by a factor of ~1.4.

**Conclusions:** Our results reassuringly demonstrate that the prospects for eugenic application of PGS are relatively limited for the foreseeable future. Given that the upper limit on SNP heritability for cognitive ability is ~0.3 (at most), and that sample sizes in the hundreds of thousands have yielded out-of-sample prediction $R2 < 5\%$, it is unlikely that attainable sample sizes in the foreseeable future will yield PGS that permit average predicted gains of more than a few IQ points at best.

**Keywords:** Cognition, Polygenic Scores, Ethics, GWAS, Prenatal

**Disclosure:** Consultant, Consultant, Self

### T167. Analysis of Polygenic Score Usage and Performance Across Diverse Human Populations

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**Background:** Studies of the relationship between genetic and phenotypic variation have historically been carried out on people of European ancestry. Efforts are underway to address this limitation, but until they succeed, the legacy of a Euro-centric bias in medical genetic studies will continue to hinder research, including the use of polygenic scores, which are individual-level metrics of genetic risk. Ongoing debate surrounds the generalizability of polygenic scores based on genome-wide association studies (GWAS) conducted in European ancestry samples, to non-European ancestry samples.

**Methods:** We analyzed the first decade of polygenic scoring studies (2008-2017, inclusive), and extracted data from 733 studies that met inclusion criteria. We also computed and analyzed polygenic scores and genetic principal components for the 2,557 1000Genomes participants.

**Results:** Two-thirds of polygenic scoring studies included exclusively European ancestry participants and another 19% included only East Asian ancestry participants. Only 3.8% of studies were carried out on samples of African, Hispanic, or Indigenous peoples. We find that effect sizes for European ancestry-derived polygenic scores are only 36% as large in African ancestry samples, as in European ancestry samples ($t=-10.056$, $df=22$, $p=5.5x10\text{-}10$). Poorer performance was also observed in other non-European ancestry samples. Analysis of polygenic scores in the 1000Genomes samples revealed many strong correlations with global principal components, and highly variable relationships between polygenic scores for height-related phenotypes, depending on methodological choices in polygenic score construction.

**Conclusions:** As polygenic score use increases in research, precision medicine, and direct-to-consumer testing, challenges posed by differential linkage disequilibrium patterns and variant frequencies across populations must be overcome in order to improve transferability of polygenic scores. These findings bolster the rationale for large-scale GWAS in diverse human populations, particularly in those that have been most underrepresented (including African ancestry, Latino, Middle-eastern, and Indigenous populations).

**Keywords:** Human Genetics, Polygenic Scores, Diversity, Molecular Genetics, Precision Medicine

**Disclosure:** Nothing to disclose.

### T168. Activation of a Hypothalamic-Ventral Tegmental Circuit Gates Motivation

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**Background:** Across species, motivated states such as food seeking, and consumption are essential for survival. Optimal performance of these behaviors is mediated by neuronal circuits that modulate energy balance and feeding. The lateral hypothalamus (LH) has been known for decades to play a fundamental role in regulating feeding and reward-related behaviors. However, the contribution of the diverse neuronal populations in the LH have not been thoroughly identified. Here we examine how lateral hypothalamic leptin receptor-expressing (LH-LEPR) neurons, a subset of GABAergic cell types in the LH, regulate motivation to obtain food.

**Methods:** Lepr-cre mice ($n = 8$ per group, mixed sex) were trained to earn food pellet reinforcers on a progressive ratio (PR) schedule of reinforcement, a widely-used behavioral task to assess motivation. A chemogenetic approach was used to selectively activate or inhibit these neurons during behavior on the PR task. Channelrhodopsin (ChR2)-assisted circuit mapping (CRACM) was used to assess synaptic connections of LH-LEPR neurons with neurons in the ventral tegmental area (VTA). An optogenetic approach was used to assess the role of LH-LEPR axonal projections to the VTA on behavior, as well as upstream inputs to the LH from arcuate hypothalamic agouti-related peptide-expressing (ARC-AGRP) neurons. Paired t-tests were used to assess results.

**Results:** Chemogenetic activation of LH-LEPR neurons significantly increased the number of food pellets earned ($p < 0.01$) as well as the number of cumulative lever presses ($p < 0.05$), while inhibition of these neurons decreased food pellets earned ($p < 0.01$) and lever presses ($p < 0.05$). CRACM revealed that LH-LEPR neurons form functional inhibitory synapses with non-dopaminergic neurons in the VTA. Optogenetic activation of these projections in vivo increased PR performance, as did optogenetic activation of ARC-AGRP axonal terminals in the LH.

**Conclusions:** These results identify LH-LEPR neurons as a new integrator of the hypothalamic-ventral tegmental circuitry that gates motivation.

**Keywords:** Motivation, Feeding, Optogenetics, Chemogenetics, Lateral Hypothalamus

**Disclosure:** Nothing to disclose.

### T169. Modulation of Incentive Motivation by Ovarian Hormones in Female Rats

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**Background:** In females, naturally occurring elevations in estradiol reduce food intake and estradiol treatment in ovariectomized rats is sufficient to reduce food intake and body weight. Stimuli paired with food (food cues) also influence feeding behavior, e.g., by increasing incentive motivation and the amount of food consumed. However, how ovarian hormones influence incentive motivational responses to food cues is unknown. Therefore here, we determined how motivation for food and food-seeking triggered by a food cue are influenced by the cycle and by ovarian hormones. Studies were conducted in female Sprague Dawley and selectively bred obesity-prone and obesity-resistant rats because obesity-prone rats show stronger incentive motivational responses to food- and drug-cues compared to obesity-resistant rats.

**Methods:** Motivation for sucrose was determined using instrumental responding and progressive ratio testing, while Pavlovian conditioned approach was used to examine incentive motivational responses to food cues. Naturally cycling rats were tested during each phase of the cycle. A separate set of rats were ovariectomized and tested for conditioned approach following singe or repeated hormone replacement (two injections of 17β-estradiol benzoate separated by 24 h, followed by one injection of progesterone, s.c.), or treatment with 17β-estradiol benzoate or progesterone alone.

**Results:** We found that female obesity-prone but not obesity-resistant rats, show greater conditioned approach during phases of the estrous cycle where estradiol is low (metestrus/diestrus) compared to phases where estradiol is high (proestrus/estrus). However, in both groups break points for sucrose were lower during proestrus and estrus compared to metestrus and diestrus. Thus, in obesity-prone rats, cue-triggered food-seeking and consumption were similarly modulated by the cycle, whereas consumption but not food-seeking was affected in obesity-resistant females. Furthermore, repeated hormone replacement was sufficient to reduce incentive-motivation in ovariectomized outbred and obesity-prone females. In contrast, administration of 17β-estradiol benzoate or progesterone alone were insufficient to reproduce this effect.

**Conclusions:** To our knowledge, this is the first demonstration that ovarian hormones modulate incentive motivational responses to food cues and motivation for sucrose itself. In addition, we find that effects of the cycle on cue-triggered food-seeking are absent in obesity-resistant females. This is consistent with reduced incentive motivational responses to food cues in obesity-resistant vs. obesity-prone males and shows a dissociation between hormonal regulation of motivation for food and food-seeking triggered by food cues. These data have implications for hormonal regulation of reward seeking in the context of obesity, addiction, and disordered feeding behavior.

**Keywords:** Ovarian Hormones, Females, Behavior

**Disclosure:** Nothing to disclose.

### T170. Characteristics of Patients With Tardive Dyskinesia Who Maintain Treatment Response After Discontinuing Long-Term Valbenazine: Pooled Analysis of Two Trials

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**Background:** Tardive dyskinesia (TD), an involuntary hyperkinetic movement disorder that is often debilitating, is associated with prolonged treatment with antipsychotics and other dopamine-receptor blocking agents. Evidence indicates that TD typically follows a fluctuating and often persistent course. However, with the advent of an approved medication (i.e., valbenazine) that reduces the severity of TD movements, more research is needed to assess if certain patients may not require sustained treatment. The long-term effects of once-daily valbenazine (40 or 80 mg) on TD have been evaluated in two phase III trials in which participants received up to 48 weeks of active treatment, KINECT 3 (NCT02274558, double-blind placebo-controlled trial with blinded extension) and KINECT 4 (NCT02405091, open-label trial). In each trial, some participants maintained a response at Week 52, 4 weeks after valbenazine was discontinued. Data from the post-washout period were analyzed post hoc to explore whether certain patient characteristics may have contributed to maintenance of response.

**Methods:** Three different types of response criteria at Week 52 (after 4-week washout) were defined as follows: ≥ 50% improvement from baseline in the Abnormal Involuntary Movement Scale (AIMS) total score (sum of items 1-7); a score of 1 ("very much improved") or 2 ("much improved") on the Clinical Global Impression of Change-Tardive Dyskinesia (CGI-TD); or a similarly defined score of 1 or 2 on the Patient Global Impression of Change (PGIC). For each definition of Week 52 response, baseline data were pooled from participants in KINECT 3 and KINECT 4 and analyzed descriptively. The same analyses were conducted in participants who did not meet the response criteria at Week 52 (AIMS < 50% improvement, CGI-TD score ≥ 3, PGIC score ≥ 3). Baseline data included: demographics, psychiatric diagnosis, age at TD diagnosis, TD severity (AIMS total score), and antipsychotic use (prior and current).

**Results:** At Week 52 (after 4-week washout), 178 participants had an available AIMS or CGI-TD assessment and 176 had an available PGIC assessment. The number of participants in each response subgroup were as follows: AIMS ≥ 50% (n = 46); CGI-TD ≤ 2 (n = 58); PGIC ≤ 2 (n = 99). In all of the Week 52 responder subgroups, approximately 50% of participants were male and the mean age was around 60 years; mean age at TD diagnosis was approximately 50 years. Schizophrenia/schizoaffective disorder was the primary diagnosis in the majority of Week 52 responders: AIMS ≥ 50%, 65.2%; CGI-TD ≤ 2, 65.5%; PGIC ≤ 2, 63.6%. Mean AIMS total score at baseline was similar across all responder subgroups: AIMS ≥ 50%, 12.5; CGI-TD ≤ 2, 12.3; PGIC ≤ 2, 13.1. Similar demographics and disease characteristics were found in participants who did not meet the responder criteria at Week 52 (AIMS < 50%, n = 132; CGI-TD ≥ 3, n = 120; PGIC ≥ 3, n = 77). In terms of antipsychotic use, prior treatment with only atypical antipsychotic(s) was similar between participants who met Week 52 response criteria (AIMS ≥ 50%, 71.7%; CGI-TD ≤ 2, 70.7%; PGIC ≤ 2, 69.7%) and those who did not (AIMS < 50%, 70.5%; CGI-TD ≥ 3, 70.8%; PGIC ≥ 3, 72.7%). Approximately 70% of Week 52 responders and non-responders were concomitantly treated with only atypical antipsychotic(s) during the trial. Based on clinician-rated outcomes, a higher percentage of Week 52 responders had no concomitant antipsychotic use during the trials (AIMS ≥ 50%, 15.2%; CGI-TD ≤ 2, 17.2%) than non-responders (AIMS < 50%, 12.9%; CGI-TD ≥ 3, 11.7%). However, for the patient-reported outcome, no concomitant antipsychotic use was more common in patients who did not meet the response definition (PGIC ≥ 3, 15.6%) than those who did (PGIC ≤ 2, 12.1%).

**Conclusions:** Based on the current results, no factors seemed to definitively predict which patients maintained a response after discontinuing long-term treatment with once-daily valbenazine. More analyses, including trials with longer withdrawal periods, are needed to understand why some patients may experience continued TD response even after valbenazine discontinuation.

**Keywords:** Valbenazine, Tardive Dyskinesia, Movement Disorders

**Disclosure:** Neurocrine Biosciences, Inc., Consultant, Neurocrine Bioscienes, Inc., Grant, TEVA Pharmaceuticals, Consultant

### T171. Long-Term Effects of Valbenazine on Tardive Dyskinesia in Patients With Schizophrenia/Schizoaffective Disorder or Mood Disorder: Results From an Open-Label, Rollover Study

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**Background:** Patients treated with antipsychotics, regardless of primary psychiatric diagnosis, are at risk for developing tardive dyskinesia (TD), a persistent and potentially debilitating movement disorder. Valbenazine is a highly selective vesicular monoamine transporter 2 (VMAT2) inhibitor approved for the treatment of TD in adults. It has been evaluated in several clinical trials including randomized controlled trials (KINECT, KINECT 2, KINECT 3), a long-term extension study (KINECT 3 extension), and a 1-year open-label study (KINECT 4). Participants who completed KINECT 3 or KINECT 4 were eligible to participate in the current rollover study (NCT02736955). Data from this study were analyzed to evaluate the long-term safety and effectiveness of once-daily

valbenazine in adults with schizophrenia/schizoaffective disorder (SZD) or mood disorder (MD).

**Methods:** Key eligibility criteria included: age 18 to 85 years; completion of KINECT 3 extension or KINECT 4; maintenance medications (for schizophrenia, schizoaffective disorder, or mood disorder) at stable doses; psychiatrically stable (Brief Psychiatric Rating Scale score < 50); and no active suicidal ideation or behavior per the Columbia-Suicide Severity Rating Scale. Following washout of prior valbenazine treatment (Week 48 to 52 of KINECT 3 and KINECT 4), participants were re-initiated at 40 mg for 4 weeks and escalated to 80 mg based on the investigators' assessments of safety/tolerability and clinical assessment of TD; a subsequent reduction to 40 mg was allowed if 80 mg was not tolerated (80/40 mg group). Participants received valbenazine for up to 72 weeks or until valbenazine became commercially available. TD response was assessed in psychiatric diagnosis subgroups (SZD, MD) using the Clinical Global Impression of Severity-TD (CGIS-TD: range, 1 "normal, not at all ill" to 7 "extremely ill") and Patient Satisfaction Questionnaire (PSQ: range, 1 "very satisfied" to 5 "very dissatisfied"). Analyses included: CGIS-TD mean scores, percentage of participants with a CGIS-TD score ≤ 2 ("normal, not at all ill" or "borderline ill"); and PSQ score ≤ 2 ("very satisfied" or "somewhat satisfied"). Safety assessments included monitoring of treatment-emergent adverse events (TEAEs). All outcomes were analyzed descriptively.

**Results:** Of 160 participants in the analyses, 104 had a diagnosis of SZD (40 mg, n = 23; 80 mg, n = 75; 80/40 mg, n = 6) and 56 had a diagnosis of MD (40 mg, n = 12; 80 mg, n = 42; 80/40 mg, n = 2). Approximately one-third of participants in each diagnosis subgroup reached the Week 48 visit (SZD: n = 34; MD: n = 22). Few reached Week 60 (SZD, n = 1; MD, n = 3) and none reached Week 72 because valbenazine became commercially available during the study. Reasons for discontinuation before study termination were as follows: SZD (withdrawal of consent, n = 5; death, n = 4 [all unrelated to treatment]; adverse event, n = 3; non-compliance, n = 3; sponsor/investigator decision, n = 1; lost to follow-up, n = 1); and MD (withdrawal of consent, n = 3; adverse event, n = 2; sponsor/investigator decision, n = 1). In participants who were maintained on valbenazine 40 mg or escalated to 80 mg (with no dose reduction), mean CGIS-TD score changes from baseline to Week 48 indicated sustained global improvement both in the SZD subgroup (40 mg, -1.1; 80 mg, 1.3) and MD subgroup (40 mg, -1.5; 80 mg, -2.9). The percentage of participants with a CGIS-TD score ≤ 2 increased from baseline to Week 48 in the SZD subgroup (baseline: 40 mg, 8.7%; 80 mg, 14.7%; Week 48: 40 mg, 37.5%; 80 mg, 60.9%) and MD subgroup (baseline: 40 mg, 0%; 80 mg, 24.4%; Week 48: 40 mg, 50.0%; 80 mg, 93.8%). At baseline, most or all participants had a PSQ score ≤ 2 in the SZD subgroup (40 and 80 mg, 100%) and the MD subgroup (40 mg, 100%; 80 mg, 97.6%), indicating satisfaction with their prior valbenazine experience in KINECT 3 or KINECT 4. At Week 48, participants continued to express satisfaction with treatment (SZD: 40 mg, 100%; 80 mg, 95.7%; MD: 40 and 80 mg, 100%). The incidence of TEAEs in all valbenazine-treated patients was similar between diagnosis subgroups (SZD, 51.0%; MD, 57.1%). Less than 10% of all participants in either subgroup discontinued due to TEAEs (SZD, 6.7%; MD, 3.6%), and none were due to worsening depression or suicidal ideation.

**Conclusions:** Valbenazine was generally well tolerated and no new safety signals were observed. Clinician-based assessments indicated ongoing and meaningful TD improvements regardless of primary psychiatric diagnosis in participants who received long-term valbenazine treatment in the current study following up to 48 weeks of treatment in previous valbenazine studies. Patient satisfaction rates with valbenazine remained high.

**Keywords:** Valbenazine, Tardive Dyskinesia, Movement Disorders

**Disclosure:** Neurocrine Biosciences, Inc., Employee

## T172. Pharmacological Effects on Interhemispheric Signal Propagation in the Motor Cortex: A TMS-EEG Study

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**Background:** Interhemispheric connections across the corpus callosum may have inhibitory or excitatory effects on signal propagation. Previous transcranial magnetic stimulation (TMS) research suggests that transcallosal fibers act to inhibit the propagation of activity between homologous brain regions; however, transcallosal disinhibition occurs in the presence of a TMS paradigm related to GABAB receptor-mediated inhibitory neurotransmission. This suggests that interhemispheric information transfer may be mediated by GABAB receptor activation. To date, no study has assessed the pharmacological modulation of interhemispheric connectivity from motor cortical stimulation. We investigated these neurochemical mechanisms by conducting a randomized, double-blind, controlled within-subjects study measuring TMS-induced interhemispheric signal propagation (ISP) with electroencephalography (EEG) recordings under the effects of baclofen, L-DOPA, dextromethorphan, and rivastigmine. We hypothesized that the administration of GABAB receptor agonist baclofen would decrease ISP when compared against placebo, thus leading to greater interhemispheric inhibition.

**Methods:** Twelve healthy volunteers (mean age: 31.3 ± 10.5 years; three females) each underwent five sessions of single pulse TMS in a random order. Sessions were preceded by the administration of placebo or the active drugs baclofen (50 mg), dextromethorphan (150 mg), L-DOPA (100 mg), or rivastigmine (3 mg). TMS-EEG measurements were conducted pre- and post-drug, with post-drug measures recorded after the drugs reached peak plasma levels. All data collection and analysis were performed while blinded to drug assignment. EEG data was analyzed using a custom script based on previous work in MATLAB. ISP was calculated by dividing the area under the rectified TMS-evoked potential (TEP) from the right motor cortex by the area under the rectified TEP from the left motor cortex. We accounted for the time it takes for transcallosal signal propagation by setting the interhemispheric transfer time to 10 ms.

**Results:** As data analysis is still underway, we are only able to discuss the results from the baclofen and placebo groups at this time. Activation of the right motor cortex was significantly lower than activation in the left motor cortex for all conditions ($t(45) = 5.989$, $p < .001$) during left motor cortex stimulation. Mixed ANOVA revealed a trending interaction between Time and Drug Type on ISP ($F(1,20) = 3.768$, $p = 0.066$). Further post-hoc comparisons with Bonferroni correction showed a significant decrease in ISP under baclofen ($t(11) = 3.018$, $p = .024$), but no change across the placebo condition ($t(10) = -.437$, $p = .670$).

**Conclusions:** Together these results suggest that baclofen reduces the amount of TMS-induced signal propagation across the corpus callosum. This provides evidence that interhemispheric inhibition is mediated by a population of interneurons involved with GABAB activity. Although we will continue to evaluate changes in ISP due to other drug effects, these findings are a fundamental step in identifying the neurochemical mechanisms involved in transcallosal connectivity.

**Keywords:** Corpus Callosum, Neuropharmocology, Transcranial Magnetic Stimulation, Electroencephalography

**Disclosure:** Nothing to disclose.

## T173. Role of PSD95 and nNOS Interaction in Regulation of Conditioned Fear and Implications for Treatment of PTSD

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**Background:** Stimulation of N-methyl-D-aspartic acid receptors (NMDARs) increases calcium-dependent production of nitric oxide (NO) by nitric oxide synthase (nNOS) and is crucial for fear memory formation. Antagonism of NMDAR and NOS activity disrupts fear conditioning but produces adverse side effects. We hypothesized that disrupting protein-protein interaction between nNOS and postsynaptic density protein 95 (PSD95) would reduce NO production that underlies fear conditioning while minimizing non-specific effects.

**Methods:** Co-immunoprecipitation, electrophysiology, behavioral paradigms, and RNA sequencing were utilized to investigate PSD95/nNOS binding and neuronal network properties in various brain regions. ZL006 and AN253 were used as tools to disrupt PSD95/nNOS binding. Behavioral effects were compared to NMDA receptor antagonist MK801.

**Results:** Immediately following cue-induced fear conditioning, association of PSD95 and nNOS is enhanced in the basolateral amygdala (BLA) and ventral hippocampus (vHP) but not in the medial prefrontal cortex (mPFC). Systemic treatment with ZL006 prevented these associations and attenuated fear memory. ZL006 treatment directly targeting the BLA by cannulae injection also attenuated cue-induced fear memory. However, treatment directly targeting the vHP had no effect on cue-induced fear memory, but impaired context induced fear memory. Cue-induced fear conditioning induced unique pattern of gene expression in the BLA. Recordings from acute brain slices revealed ZL006 or AN253 prevent induction of BLA long-term potentiation in naïve animals and prevent increases to BLA excitatory neurotransmission in fear conditioned animals.

**Conclusions:** PSD95/nNOS interaction at specific sites within the fear network is a key step in acquisition of conditioned fears. Disrupting PSD95/nNOS protein-protein interaction represents a novel treatment approach for fear-related disorders such as post-traumatic stress disorder (PTSD).

**Keywords:** PTSD, Amygdala, Neuronal Nitric Oxide Synthase, Long Term Potentiation, Fear Conditioning

**Disclosure:** Contracted Research, Grant

## T174. Dopamine Transporter (DAT1) Gene in Combat Veterans With PTSD

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**Background:** Posttraumatic stress disorder (PTSD) is a chronic and debilitating psychiatric condition. There is a 40% variance in PTSD accounted for by genetic factors. Researchers who have been studying PTSD are interested in dopamine given its role in attention, fear and stress response and implications in PTSD psychopathology. Dopamine transporter gene, DAT1, is of particular focus which encodes dopamine transporter. Dopamine transporter is postulated to regulate synaptic dopamine concentration.DAT1 (SLC6A3) gene has a 40 base pair variable number tandem repeat (VNTR) with 3-11 copies. The two most common alleles of DAT1 VNTR polymorphisms are: 10 and 9-Repeats (9 R). DAT1 has been studied for PTSD in general US community. However, there has not been a DAT1 candidate gene study in veterans. We hypothesize that there will be a statistically significant association between DAT1 polymorphisms and PTSD in a case-control combat exposed veteran population of 664.

**Methods:** Participants were recruited through the Veteran Affairs centers at Cincinnati, OH and Charleston, SC; each participant had a semi-structured diagnostic interview. Inclusion criteria included history of combat exposure and Combat Exposure Scale (CES) score above 10. Exclusion criteria included current or lifetime DSM-IV diagnoses of bipolar or primary psychotic disorder. Clinician Administered PTSD scale (CAPS) was used for PTSD assessment. Genotyping were performed by a laboratory assistant and Z.W who were blinded to case-control status and other clinical assessments. The Hardy-Weinberg equilibrium (HWE) was verified prior to any formal analysis. Multivariable logistic regression and linear regression were utilized to identify predictors associated with PTSD and CAPS (along with its subscales).

**Results:** There were 299 Combat exposed veteran controls and 365 PTSD cases with 84% were men and 71% were Caucasians in total sample. Baseline measures were statistically different between the two groups: PTSD cases were average 3 years younger than controls (43.9 y + /-14.2 versus 46.8 y + /-13.8, $p = 0.011$) and more African Americans were in the case group than control (26.8% versus 16.4%, $p = 0.003$). Genotype 9 R allele was predictive of PTSD diagnosis (OR: 1.6, 95% CI: 1.049-2.44, $p = 0.029$).

**Conclusions:** We report the first DAT1 gene association study in veterans for PTSD. We also confirmed prior findings in general population of DAT1 9 R allele association with PTSD. Our study design on homogenous trauma type, combat, is an improvement over prior trauma and PTSD studies. Our results will need to be confirmed in future veteran studies.

**Keywords:** PTSD, Dopamine Transporter, Combat Veteran

**Disclosure:** Nothing to disclose.

## T175. Machine Learning Multivariate Pattern Analysis Predicts Classification of Posttraumatic Stress Disorder and its Dissociative Subtype

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**Background:** Recently, a growing number of studies have applied machine learning methods to neuroimaging data in order to predict and characterize psychiatric disease, which in turn may help to facilitate the early identification of heterogeneous subtypes of illness. Critically, these techniques have not been utilized to predict subtypes of posttraumatic stress disorder (PTSD), including the dissociative subtype of PTSD (PTSD + DS).

**Methods:** Using Multiclass Gaussian Process Classification (MGPC) within PRoNTo software, we examined the classification accuracy of: i) neural activation during the resting-state [mean amplitude of low-frequency fluctuations (mALFF)]; and ii) seed-based amygdala complex resting-state functional connectivity, within 181 participants [PTSD ($n = 81$); PTSD + DS ($n = 49$) and age-matched healthy trauma-unexposed controls ($n = 51$)]. Additionally, in order to compare regional group differences, we also computed mass-univariate analyses on resting-state activation and functional connectivity measures (FDR-cluster corrected $p < .05$, $k = 20$).

**Results:** Extracted features from the machine learning analysis could predict accurately the classification of PTSD, PTSD + DS, and healthy controls, using both resting-state activation (91.63% balanced accuracy, $p < .001$) and amygdala complex connectivity maps (85.00% balanced accuracy, $p < .001$). These results were replicated using independent machine learning algorithms/cross-validation procedures. Univariate analyses within SPM revealed that the PTSD + DS group displayed significantly increased activation within emotion regulation regions, whereas the PTSD group showed increased activation within the amygdala, globus pallidus, and motor/somatosensory regions. This was on balance with areas weighted as being most important for group classification within the machine learning analysis.

**Conclusions:** In order to optimize care, the field of psychiatry would benefit significantly from the early identification of objective biomarkers that could predict heterogeneous subtypes of illness as well as individual patient symptom trajectories. The results of the current study have significant implications for advancing machine learning applications within the field of psychiatry, as well as for developing objective biomarkers indicative of diagnostic heterogeneity.

**Keywords:** Posttraumatic Stress Disorder, Machine Learning, Functional MRI (fMRI), Amygdala

**Disclosure:** Nothing to disclose.

## T176. The Impact of Prenatal Exposure to Infection on Alcohol Drinking Behavior and Brain Reward Circuit Activity

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**Background:** Prenatal exposure to infection is a risk factor for many neuropsychiatric disorders, including depression and schizophrenia, which are often comorbid with substance use disorders. Such comorbidity exacerbates the underlying neuropsychiatric disorder. Green and colleagues have proposed that reduced connectivity in the brain reward circuit (BRC) may underlie enhanced vulnerability to substance use disorders in patients with schizophrenia, and studies of patients with schizophrenia and co-occurring cannabis use disorder have demonstrated a hypoconnected BRC (using resting-state fMRI connectivity). Interestingly, the neonatal ventral hippocampal lesion (NVHL) rat, an animal model of schizophrenia, if exposed to alcohol as an adolescent, demonstrates increased alcohol consumption in adulthood. Our lab has also observed that in adulthood these animals demonstrate reduced connectivity between the ventral striatum and cortical regions. Few studies, however, have investigated the impact of prenatal exposure to infection on alcohol drinking behavior and BRC function in adult offspring. The technique of maternal immune activation (MIA) is a well-characterized method of inducing prenatal exposure to infection in rodents, and offspring demonstrate multiple behavioral and neurobiological phenotypes reflective of neuropsychiatric illness. In the current study, we assessed alcohol drinking behavior, as well as activity within the BRC (through measurement of local field potentials [LFPs]), in adult rats exposed to prenatal infection via MIA.

**Methods:** Pregnant dams were injected intravenously with polyinosinic:polycytidylic acid [poly(I:C); 4 mg/kg] or saline (1 mL/kg) on gestational day 15. Poly(I:C) is a synthetic analog of double-stranded RNA, which leads to a heightened immune response in rats. In adulthood, male and female rats exposed to prenatal infection (MIA rats) and control rats (offspring of saline injected dams) were separated into two cohorts. Cohort 1 ($N = 10$/group) was trained to drink 10% alcohol using a sucrose-fade technique in their home cage for 90 minutes a day, 5 days per week (M-F). Once drinking 10% alcohol without sucrose, rats continued to drink for 4 weeks. Area under the curve was calculated for grams of alcohol consumed per kg of body weight (g/kg) across all sessions and used for statistical analysis. Cohort 2 ($N = 10$/group) was implanted with electrodes targeting regions of the BRC (specifically the bilateral nucleus accumbens shell [NacSh], infralimbic [IL] and prelimbic [PL] medial prefrontal cortex, and the CA1 region of the hippocampus). Following recovery, LFPs were recorded from each awake, freely-behaving rat during two 30-minute sessions. All data from each 30-minute recording were analyzed using established frequency ranges (delta = 1-4 Hz, theta = 5-10 Hz, alpha = 11-14 Hz, beta = 15-30 Hz, low gamma = 45-65 Hz, and high gamma = 70-90 Hz) from the rodent literature. Standard LFP signal processing to characterize the power spectral densities within, and coherence between brain regions for each rat was calculated using custom code written for Matlab R2017a. Using the machine-learning algorithm lasso, we then built predictive models to classify rats based on prenatal exposure (MIA vs. control) and compared the model performance from real data to the performance of models built and tested on data permutations. We also identified neural features that were significantly different between groups by performing t-tests on each feature and using an FDR correction to adjust for multiple comparisons.

**Results:** A univariate ANOVA indicated that while MIA rats tended to drink less alcohol than controls, there was only a trend-level effect of group on g/kg of alcohol consumed [$F(1,18) = 4.04$, $p = 0.06$, $np2 = 0.18$]. In Cohort 2, three animals were removed from the study (one MIA and two controls) due to electrode failure. For the LFP data, models built from the real data were able to predict which rats were MIA and which rats were controls better than models built from the permuted data (65.88% vs. 51.67%; Cohen's $d = 0.92$). We were subsequently able to determine the neural features that were significantly different between groups. MIA rats showed higher right PL delta and theta power, as well as higher delta coherence between the left CA1 and left NAcSh, higher delta coherence between the left CA1 and right PL, and higher alpha coherence between the left PL and right IL ($p = 0.05$ for each feature).

**Conclusions:** Unlike what we and others have observed regarding alcohol drinking in the NVHL rat model (and what has been reported regarding the increased rate of alcohol use disorders in patients with schizophrenia), MIA rats did not demonstrate an increase in alcohol consumption. However, MIA rats did have altered BRC function as assessed by LFPs - specifically increased power in cortical regions, as well as hyperconnectivity between the hippocampus and cortical regions, and the hippocampus and ventral striatum. These preliminary data, thus, do not allow us to confirm whether the MIA rat can serve as a model of neuropsychiatric illness with co-occurring substance use. It may be necessary, however, to expose MIA rats to alcohol during adolescence (as is necessary for the NVHL rats) in order to induce increased alcohol drinking in adulthood. Further studies will be required to fully characterize the nature of both alcohol/substance use behaviors and neural connectivity within components of the BRC in the MIA rat.

**Keywords:** Local Field Potentials, Alcohol, Maternal Immune Activation, Reward Circuitry

**Disclosure:** Nothing to disclose.

## T177. Loss of Disrupted in Schizophrenia 1 Contributes to Sex-Specific Deficits in Neurite Density and White Matter Integrity

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**Background:** Diffusion tensor imaging (DTI) has provided remarkable insight into our understanding of white matter microstructure and brain connectivity across a broad spectrum of psychiatric disease. While DTI and other diffusion weighted magnetic resonance imaging (MRI) methods have clarified the axonal contribution to the disconnectivity seen in numerous psychiatric diseases, absent from these studies are quantitative indices of neurite density and orientation that are especially important features in regions of high synaptic density that would capture the synaptic contribution to the psychiatric disease state. Whereas quantitative indices of DTI such as fractional anisotropy (FA) are able to capture microstructural features but are inherently nonspecific, multi-compartment diffusion techniques such as NODDI are able to model water diffusion across multiple compartments, thus enabling more granular microstructural information that are important features in these regions of higher synaptic density. Here we report the application of neurite orientation dispersion and density imaging (NODDI) to a novel Disc1 knockout rat model of psychiatric illness.

**Methods:** To generate the Disc1 knockout, the N-terminal second coding exon of Disc1 was selected for targeting via CRISPR/Cas9. Two highly specific target sequences were utilized to generate a knockout allele that could be followed with simple PCR. Fertilized Sprague-Dawley zygotes were injected with CRISPR/Cas9 reagents, and injected embryos were transferred into pseudopregnant surrogate mothers. A founder carrying an early translational stop codon at amino acid position 68 was then backcrossed to outbred Sprague-Dawley rats to generate a Disc1-/- colony. Ex vivo DTI imaging ($N = 6$ Disc1-/- male, 6 Disc1-/- female, 7 wild-type male, 6 wild-type female) was conducted with a 4.7-T Agilent MRI system with multi-slice, diffusion-weighted, spin echo images. DTI-TK provided a study-specific tensor template to which each subject tensor volume was then spatially normalized. An FA threshold of 0.2 was applied for the creation of the skeleton, corrected for multiple comparisons and threshold-free cluster enhancement, with $p < .05$ as threshold for significance. For NODDI, volumetric parameter maps were constructed and output volumes include neurite density, orientation dispersion index, and CSF volume fraction. For prepulse inhibition testing, startle chambers containing a Plexiglas cylinder resting inside a sound-attenuating cabinet with a speaker to produce acoustic stimuli were used. The startle response to the onset of the 120-dB burst was recorded for 100 ms for each pulse-alone trial, prepulse + pulse trial, and from the onset of each no stimulus trial. Two measurements (startle magnitude and %PPI) were calculated from these values for each rat for each of the different treatment conditions ($N = 15$ Disc1-/- male, 15 Disc1-/- female, 21 wild-type male, 18 wild-type female).

**Results:** To explore and characterize the influence of Disc1 on white matter microstructure, ex-vivo whole-brain DTI was performed. Whole-brain voxel-wise tract-based spatial statistics (TBSS) analysis comparing our Disc1-/- model to age and sex-matched controls were performed at postnatal day 84 (P84). Disc1-/- male rats demonstrate decreased FA mainly in the left superior neocortex, external capsule, corpus callosum, internal capsule, and left amygdala when compared to matched controls. Sex-specific differences in the distribution of voxel-wise changes in measures of the diffusion tensor are also evident with lower FA values identified in the left inferior neocortex, external capsule, corpus callosum, and internal capsule of female Disc1-/- rats when compared to matched controls. To further explore the role of Disc1 on neural structure and organization, ex-vivo whole-brain NODDI was also employed. TBSS analysis uncovered confluent areas of decreased neurite density index (NDI) and orientation dispersion index (ODI) in the Disc1-/- model. Disc1-/- males displayed decreased NDI values in the inferior neocortex, external capsule, and right amygdala when compared with matched controls. Decreased ODI was seen spread over a large portion of the superior neocortex, external capsule, and corpus callosum. Disc1-/- female rats demonstrated decreased NDI in the right superior neocortex, external capsule, corpus callosum, and right internal capsule. Disc1-/- animals did not demonstrate significant differences in prepulse inhibition (PPI) when compared to control animals.

**Conclusions:** We present a homozygous Disc1 knockout rat model of psychiatric illness and demonstrate the significant impact the loss of Disc1 imparts along multiple quantitative neuroimaging measures of neural structure. Our neuroimaging studies reveal and clarify both the axonal contributions (DTI) and synaptic contributions (NODDI) to the psychiatric disease state and quantitatively capture the microstructural changes that accompany an important gene variant implicated in several psychiatric illnesses. The Disc1 knockout model emerges as a natural and convenient entry point to explore the neurostructural features of a loss of function model with implications across several psychiatric illnesses and concomitantly, with the generation of a homozygous Disc1-/- animal model, provide a new platform to explore the biological role of Disc1 in the neuropathogenesis of psychiatric illness.

**Keywords:** Disrupted-in-Schizophrenia 1, Diffusion Tensor Imaging (DTI), NODDI, Animal Models

**Disclosure:** Nothing to disclose.

## T178. Toward Understanding a Mouse Model of Schizophrenia Using CaMKIIa Heterozygous Knockout Mouse: From Cellular and Behavioral Phenotypes to a Biomarker of Pathophysiology

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**Background:** Animal models of schizophrenia that present not only pathophysiology but also a wide array of symptoms affected in the patients are an urgent need. Mounting evidence from human genetic studies indicate an involvement of gene networks related to NMDA receptor/CaMKIIa signaling. Recently, we generated a mouse line constitutively lacking CaMKIIa gene and reported a cellular feature, immature dentate gyrus, characterized by increased number of immature neuronal progenitors and a concomitant decrease in mature neurons in hippocampus. More importantly, immature dentate gyrus was detected in postmortem brains of a subset of schizophrenia and bipolar patients, raising a possibility that this cellular phenotype may present underlying pathophysiology of schizophrenia.

**Methods:** Using CaMKIIa-heterozygous knockout (hKO) mice, we conducted a broad array of behavioral tests relevant to symptoms in schizophrenia. This included locomotor, elevated-zero maze test, novelty object recognition test, context-dependent fear conditioning, social interaction, and sucrose preference. In an attempt to identify a biomarker specific to a

behavioral deficit, electroencephalogram (EEG) recording was also carried out. Using Spike 2 software, a recording script, inter-stimulus interval, was generated, which is composed of 5 blocks of intervals (0.5, 1, 2, 4, and 8 sec) and each block contains 200 single click.

**Results:** CaMKIIa-hKO mice were hypoactive in-home cage environment and presented less anxiety-like behavior. Furthermore, deficits in cognitive function were prominent in CaMKIIa-hKO mice as assessed in novelty object recognition test as well as short and long-term memory formation in fear conditioning paradigm. However, behavioral phenotypes related to negative symptoms including sociability and anhedonia-like behavior were similar to those observed in wild type littermate. Based on the cognitive impairments as well as immature dentate gyrus observed in CaMKIIa-hKO mice, we recorded EEG from an electrode implanted in CA3 subregion of hippocampus at resting state as well as in response to click sound stimuli. CaMKIIa-hKO mice showed an increase in auditory-evoked high-frequency gamma power although baseline gamma was unchanged. Additionally, some characteristics of event-related potentials were altered.

**Conclusions:** Collectively, CaMKIIa-hKO mice displayed some of the behavioral and cellular features reminiscent of schizophrenia. EEG profile suggests this mouse line potentially provides a unique opportunity to lay out a therapeutic strategy from the point of patient stratification as well as proof of pharmacology.

**Keywords:** CaMKII, EEG, Behavior, Cognitive Impairments

**Disclosure:** Astellas Research Institute of America LLC, Employee

### T179. DNA Methylation and Transcription of the Synapse-Related Genes BAIAP2 and DLG1 are Altered and Correlated With Dendritic Spine Density in the Superior Temporal Gyrus of Subjects With Schizophrenia

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**Background:** Reduced dendritic spine density (DSD) in the cerebral cortex is among the most consistently observed abnormalities in postmortem studies of schizophrenia (SZ). The molecular mechanisms giving rise to this key SZ intermediate phenotype are poorly understood, and their elucidation holds promise for understanding SZ pathophysiology and identifying SZ treatment targets. In an earlier study of postmortem gray matter from the superior temporal gyrus (STG) using the Illumina Human Methylation 450 K Array, we demonstrated that DNA methylation (DNAm) correlated with DSD at more genomic sites than expected by chance in non-psychiatric control (NPC) subjects, and this relationship was disrupted in subjects with SZ. Based on data from this genome-wide study of DNAm-DSD correlations, we identified two synapse-related genes to study in greater depth: brain-specific angiogenesis inhibitor 1-associated protein 2 (BAIAP2) and discs large, drosophila, homolog of, 1 (DLG1). BAIAP2 is a regulator of the actin cytoskeleton in dendritic spines, and DLG1 is involved in the trafficking of glutamate receptors to the post-synaptic membrane. Here, we measured BAIAP2 and DLG1 transcript abundance and investigated the relationship of transcript abundance to local DNAm and DSD in postmortem tissue from the STG of subjects with SZ and NPC subjects.

**Methods:** We used targeted bisulfite sequencing and quantitative PCR to characterize BAIAP2 and DLG1 DNAm and transcript abundance in postmortem gray matter from the STG of subjects with SZ and matched NPC subjects in which DSD had previously been characterized.

**Results:** We replicated and extended our earlier findings of strong DNAm-DSD correlations in multiple regions of BAIAP2 (promoter, introns 4 & 8) and the promoter of DLG1. We also found that abundance of all major BAIAP2 transcript variants was increased, and that abundance of all major DLG1 transcript variants was decreased, in SZ subjects relative to NPC subjects. Finally, we showed a significant negative correlation between total BAIAP2 transcript abundance and DSD.

**Conclusions:** Together, these data support altered DNAm in STG gray matter, by affecting transcription of synapse-related genes including BAIAP2 and DLG1, as a potential molecular mechanism for reduced DSD in subjects with SZ. Future studies will use CRISPR-dCas9 technology to alter DNAm in specific-regions of BAIAP2 and DLG1 in primary neuronal cultures and assessing the effects on DSD.

**Keywords:** DNA Methylation, Epigenetics, Postmortem Brain Tissue, Gene Transcription, Schizophrenia

**Disclosure:** Nothing to disclose.

### T180. Atypical Antipsychotics Differentially Dysregulate Insulin, Energy Sensing, and MAPK Pathways in Rat and Mouse Hypothalamic Neurons

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**Background:** Antipsychotics are the gold-standard treatment for schizophrenia but cause serious metabolic side-effects. The hypothalamus is the primary brain region responsible for energy regulation, and disruptions in energy sensing and insulin signaling in the hypothalamus are implicated in insulin resistance and obesity. Thus, dysregulation of the hypothalamus could be involved in antipsychotic-induced metabolic disturbances, yet direct effects of antipsychotics on the hypothalamus have yet to be examined.

**Methods:** The immortalized hypothalamic cell lines, rHypoE-19 (rat) and mHypoE-46 (mouse), were treated with the antipsychotics olanzapine, clozapine, or aripiprazole, with or without insulin stimulation. Western blotting was used to measure the energy sensing protein AMPK, components of the insulin signaling pathway (AKT, GSK3B), and components of the MAPK pathway (ERK1/2, JNK, p38), the latter which is also linked to inflammation.

**Results:** In the rHypoE-19 cells, olanzapine and clozapine increased pERK1/2 and pJNK, while aripiprazole increased pJNK. Clozapine and aripiprazole increased pAMPK and inhibited insulin-induced pAKT. In the mHypoE-46 cells, olanzapine and aripiprazole increased pAMPK, while clozapine and aripiprazole again inhibited insulin-induced pAKT. Clozapine also increased pJNK and aripiprazole increased pERK1/2.

**Conclusions:** Our findings suggest highly differential effects between antipsychotics on hypothalamic insulin, energy sensing, and MAPK pathways. In the rHypoE-19 neurons, upregulation of MAPK proteins by all antipsychotics suggests potential upregulation of pro-inflammatory pathways. In the rHypoE-19 and mHypoE-46 lines, aripiprazole and clozapine inhibition of insulin-stimulated pAKT and induction of pAMPK suggests impaired insulin action and energy sensing. In addition, we also found marked variances between cell type, potentially due to species or receptor expression differences. Overall, our results suggest differential and pleotropic effects of antipsychotics on the hypothalamus, which do not necessarily consistently align with

known metabolic liability of these agents (i.e. clozapine = olanzapine > aripiprazole). Our data warrants further exploration into the mechanism of these effects, including replication in an in vivo model.

**Keywords:** Atypical Antipsychotics, Hypothalamus, Insulin

**Disclosure:** Nothing to disclose.

### T181. Novel Circadian Rhythms in the Prefrontal Cortex in Schizophrenia Drive Differential Gene Expression

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**Background:** Schizophrenia (SCZ) is a debilitating psychiatric disorder that is associated with significant disturbances in cognitive control, as well as circadian rhythms and sleep. Several studies have measured changes in gene expression in SCZ subjects versus comparison subjects in various cortical regions and have identified differential expression changes in genes associated with mitochondrial function, GABAergic transmission, and immune function among others. It's thought that these expression changes might contribute to the pathophysiology of the disorder.

**Methods:** In the current study, we utilized a time-of-death analysis of RNA sequencing data from the Common Mind Consortium, and data obtained selectively from the University of Pittsburgh brain bank, to identify and compare gene expression rhythms, and genes with differential expression in the human dorsolateral prefrontal cortex (dlPFC) of SCZ subjects and comparison control subjects. We first established rhythmic genes in a cohort of 104 control subjects and then analyzed separately a matched cohort of 46 SCZ subjects and 46 comparison subjects. We employed a combination of data analysis programs and methods.

**Results:** We discovered that approximately 18% of the transcripts in the dlPFC have a diurnal rhythm and that many of these genes are similar to those identified in a microarray study from a different cortical region that we published previously (Chen et al., PNAS 2015). Interestingly, there was only a small degree of overlap between rhythmic transcripts in control and SCZ subjects. Moreover, transcripts from SCZ subjects displayed a distinct pattern of rhythmicity, with most genes showing a peak in expression during the day and a trough at night, compared to control subjects in which transcripts peaked at various times of the day. Many of the transcripts that are only rhythmic in SCZ subjects are associated with mitochondrial function, with daytime peaks in expression matching the overall expression levels of control subjects and the nighttime trough falling below this level. Moreover, many of the changes in gene expression that have been reported in SCZ vs control subjects are found only in subjects that died at night.

**Conclusions:** These data suggest that gene expression rhythms in the dlPFC of schizophrenia subjects are largely distinct from healthy controls and this results in altered transcript levels, particularly during the night. These changes at night could be driven by diurnal rhythms in neuronal activity, distinct clock-related transcriptional complexes, distinct patterns of RNA degradation or other factors. Future studies will determine the specificity of these results across brain regions, cell types and disorders, and help identify the molecular mechanisms.

**Keywords:** Circadian Rhythm, Cortical Circuit Function, Schizophrenia, Gene Expression

**Disclosure:** Nothing to disclose.

### T182. Development of GABAA Receptor Subunit Transcripts in Layer 3 Pyramidal and Parvalbumin Neurons in Monkey Visual and Prefrontal Cortices

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**Background:** Visuospatial working memory (vsWM) is a key cognitive function impaired in schizophrenia. vsWM requires information transfer among cortical regions, including visual and prefrontal cortices, via layer 3 pyramidal neurons whose activity is regulated by GABAergic parvalbumin (PV) neurons. In primates, vsWM performance improves through adolescence and certain measures mature earlier in visual cortex (V2) than prefrontal cortex (PFC). Phasic GABA neurotransmission undergoes protracted postnatal maturation in the PFC, with a progressive shift from alpha-2 (GABRA2)- to alpha-1 (GABRA1)-subunit containing GABAA receptors, which is important for neural oscillations underlying vsWM. However, whether the pattern and timing of these GABRA1 and GABRA2 messenger RNA developmental trajectories is conserved across different cells types and cortical regions has not been examined. Here we tested whether this developmental molecular shift in pyramidal and PV neurons occurs earlier in V2 than PFC. Understanding the timing of molecular changes during typical postnatal development may provide insight into how deviations from the typical trajectory could increase schizophrenia risk.

**Methods:** Rhesus monkey V2 and PFC tissue from neonatal, prepubertal, late-pubertal and adult ages was labeled by fluorescence in situ hybridization for DAPI (cell nuclei), PV (GABA neuron marker) or vGLUT1 (pyramidal neuron marker), GABRA1 and GABRA2.

**Results:** In pyramidal neurons, the GABRA1/GABRA2 ratio expression increased across all ages both in V2 and PFC. In V2, there was a significant increase in the ratio from prepubertal to late-pubertal ages. In contrast in PFC, the ratio rose significantly from prepubertal to late-pubertal, and from late-pubertal to adult ages. For PV neurons, the ratio increased across all ages. Both in V2 and PFC, the increases were similar from neonatal to prepubertal ages.

**Conclusions:** Findings for GABRA1/GABRA2 mRNA trajectories in layer 3 pyramidal neurons support the hypothesis that V2 achieves adult levels of expression earlier than PFC. However, these findings appear to be specific to pyramidal as they were not detected in PV neurons. This difference suggests that vsWM abnormalities, and possible schizophrenia risk related to GABA receptor expression levels, may be cell type-specific.

**Keywords:** Visuospatial Working Memory, GABA-A receptors, RNAscope Fluorescence in Situ Hybridization, Neurodevelopment, Cortical Circuit Function, Schizophrenia

**Disclosure:** Nothing to disclose.

### T183. Neurophysiologic Measures of Target Engagement Predict Response to Auditory-Based Cognitive Training in Treatment Refractory Schizophrenia

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**Background:** Cognitive impairment is a core feature of schizophrenia and a strong predictor of psychosocial disability. Auditory-based targeted cognitive training (TCT) aims to enhance verbal learning and other domains of cognitive functioning through "bottom-up" tuning of the neural systems underlying early auditory information processing (EAIP). Although TCT has demonstrated efficacy at the group level, individual response to TCT varies considerably, with nearly half of patients showing little-to-no benefit. EEG measures of EAIP, mismatch negativity (MMN) and P3a are sensitive to the initial TCT response and might therefore predict clinical outcomes after a full course of treatment. This study aimed to determine whether initial malleability of MMN and P3a to 1-hour of auditory-based TCT predicts improvements in verbal learning and clinical symptom reduction following a full (30-hour) course of TCT.

**Methods:** Treatment refractory patients diagnosed with schizophrenia were randomly assigned to receive treatment-as-usual (TAU; $n = 22$) or TAU augmented with TCT ($n = 23$). EEG was assessed immediately before and after the first one-hour exposure to the cognitive training exercises.

**Results:** Results indicated that malleability (i.e., change from baseline after the initial 1-hour dose of TCT) of MMN and P3a predicted improvements in verbal learning as well as decreases in the severity of positive symptoms.

**Conclusions:** Examination of MMN and P3a malleability in patients after their first dose of TCT can be used to predict clinical response to a full course of treatment and shows promise for future biomarker-informed treatment assignment.

**Keywords:** Schizophrenia, Targeted Cognitive Training, Cognitive Remediation, Mismatch Negativity, MMN

**Disclosure:** Nothing to disclose.

### T184. Long-Term Safety for Lumateperone (ITI-007) in the Treatment of Schizophrenia

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**Background:** Lumateperone (ITI-007) is an investigational drug in late phase clinical development for the treatment of schizophrenia, bipolar depression, agitation associated with dementia and other neuropsychiatric disorders. With a unique mechanism of action, lumateperone modulates serotonin, dopamine and glutamate. More specifically, lumateperone is a potent serotonin 5-HT2A receptor antagonist, a dopamine receptor phosphoprotein modulator (DPPM) acting as a presynaptic partial agonist and postsynaptic antagonist at dopamine D2 receptors, a dopamine D1 receptor-dependent indirect modulator of glutamate (both NDMA and AMPA), and a serotonin reuptake inhibitor. In two previous placebo-controlled trials, lumateperone demonstrated statistically significant improvements over placebo on change from baseline on the Positive and Negative Syndrome Scale (PANSS) total score in patients with acute schizophrenia. In an acutely ill population, lumateperone was found to be well tolerated with a safety profile similar to placebo. The purpose of the present study was to evaluate the safety of lumateperone in an open-label safety study in patients with stable schizophrenia switched from standard-of-care (SOC). The study was conducted in two parts. In the first part, patients were switched to lumateperone for 6-weeks of treatment and then switched back to SOC. In the second part, patients were switched to lumateperone for up to 1-year treatment duration.

**Methods:** To be eligible for inclusion in the study, patients must have had a clinical diagnosis of schizophrenia according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) and be stable with respect to their schizophrenia symptoms. The primary objective was to determine the safety of lumateperone, assessed by adverse events, body weight, 12-lead electrocardiograms, vital signs, clinical laboratory tests, motor assessments [Barnes Akathisia Rating Scale (BARS), Simpson-Angus Scale (SAS), and Abnormal Involuntary Movement Scale (AIMS)], and the Columbia-Suicide Severity Rating Scale (C-SSRS). The secondary objectives were to determine the effectiveness of lumateperone to maintain or improve psychopathology as measured by the PANSS and the Clinical Global Impression scale for Severity of Illness (CGI-S). Lumateperone (ITI-007 60 mg, equivalent to 42 mg lumateperone the active base) was administered orally once daily open-label in the evening with no dose titration needed.

In the first part of study, 302 patients were treated for 6 weeks with lumateperone. Following treatment, patients were switched back to SOC and reassessed approximately 2 weeks after the last dose of lumateperone.

In the second part of the study, 603 patients were treated for up to 1 year with lumateperone.

**Results:** Lumateperone was well tolerated with a favorable safety profile in patients with stable schizophrenia.

In the first part of the study, the most frequent treatment-emergent adverse event was somnolence (7.0%). Other treatment-emergent adverse events occurring in > 5% of patients were dry mouth and headache. The proportion of patients experiencing motor adverse events was low: akathisia (0.3%) and extrapyramidal side effects (0.7%). There were no treatment-emergent motor side effects as measured by the BARS, SAS or AIMS. In comparison to SOC baseline, mean body weight decreased with lumateperone treatment. Blood glucose levels remained stable. Mean change in total and LDL cholesterol, triglycerides and prolactin improved with switch from SOC baseline to lumateperone and worsened again when patients returned to SOC after two weeks of treatment. Improvements in symptoms of schizophrenia improved with lumateperone treatment as evidenced by a PANSS total score responder analysis at Day 42 showing 21.9% of patients with > 20% improvement from SOC baseline.

With long-term administration in the second part of the study, there continued to be no signals for treatment-emergent extrapyramidal side effects (EPS), including akathisia. Mean body weight decreased from SOC antipsychotic baseline with long term lumateperone treatment. Lumateperone continued to demonstrate a favorable cardiometabolic and endocrine safety profile. Mean levels of cholesterol, LDL and prolactin improved from SOC baseline with long-term lumateperone treatment and mean levels of glucose and insulin remained stable. The cardiovascular safety of lumateperone was also favorable with no QTc interval prolongation.

Importantly, stable symptoms of schizophrenia did not worsen with long-term lumateperone treatment. Although this open-label safety study was not designed to measure efficacy, improvements were observed in change from baseline of the PANSS total scores in this stable patient population switched from SOC antipsychotic therapy. The stability of psychopathology was also evident in the CGI-S scores that remained stable or improved with long-term lumateperone treatment.

**Conclusions:** Lumateperone represents a novel approach to the treatment of schizophrenia with a favorable safety profile in clinical trials. The lack of metabolic, motor and cardiovascular safety issues in both acute and long-term studies presents a safety profile differentiated from standard-of-care antipsychotic therapy. These data, taken together, are consistent with and extend data previously reported in both placebo-controlled studies in patients with acute schizophrenia with lumateperone and short-term evaluation of lumateperone's safety.

**Keywords:** Schizophrenia Novel Treatment, Long-Term Safety, Antipsychotic

**Disclosure:** Intra-Cellular Therapies, Employee

### T185. Effects of a Selective Estrogen Receptor Beta Agonist (LY500307) for Brain Target Engagement, Negative Symptoms and Cognitive Impairment Associated With Schizophrenia

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**Background:** Several lines of investigation support the potential therapeutic effects of estrogen for the negative and cognitive symptoms of schizophrenia. Women with schizophrenia have a later age of onset, a less severe course of illness, and fewer negative symptoms compared to males. Additionally, low levels of estrogen have been associated with more severe negative symptoms and cognitive deficits in females with schizophrenia. Estrogen therapy has been shown to be effective in female patients with schizophrenia for negative and cognitive symptoms. This has led to the hypothesis that estrogen may have a neuroprotective effect for schizophrenia. The effects of estrogen are mediated through two estrogen receptors – ER-alpha (ERα) and ER-beta (ERβ). Unlike ERα, ERβ is essentially absent from adult pituitary and endometrium so agonists for this receptor subtype would be less likely to be associated with risks of chemical castration or feminization effects in men and uterine cancer. Additionally, there is substantially greater ERβ than ERα receptor densities in the cerebral cortex and hippocampus, two brain regions implicated in the cognitive impairment and negative symptoms of schizophrenia. Thus, these data suggest an ERβ agonist may represent an innovative treatment for schizophrenia. We conducted the first clinical trial of a selective ERβ agonist (LY500307) in a major psychiatric disorder. The focus of this study was brain target engagement, safety and efficacy for negative and cognitive symptoms in schizophrenia.

**Methods:** A two-stage, single-site, multi-dose, phase 1a/2b adaptive design in male patients with schizophrenia was used. Subjects were recruited from the Indiana University Psychotic Disorders Program. Primary endpoints were EEG/Mismatch Negativity and fMRI/N-Back working memory for target engagement, negative (NSA-16) and cognitive (MATRICS) symptoms for efficacy, and safety (ECG indices, adverse events).

Stage 1: Subjects were randomized in double-blind, 1:1:1 fashion to placebo, 25 mg, and 75 mg daily doses. The goal of Stage 1 was to identify and advance the highest dose that did not demonstrate a safety signal and that had target selectivity for ERβ as determined by lack of total testosterone (TT) suppression and signs of feminization. If these criteria were fulfilled at both doses, the larger of the two (75 mg/day dose) would be advanced to Stage 2.

Stage 2: Subjects were randomized 1:1:1.5 to placebo, 75 mg, and 150 mg daily doses. The final data set for analyses of target engagement, efficacy, and safety analyses encompassed all randomized subjects. Dose-response was the primary analytic strategy employed.

**Results:** Ninety-five subjects were randomized in the trial (30 in stage 1, 65 in stage 2).

Sample characteristics: mean (SD) age of 37.3 (12.1) years; 56% African American/40% white Caucasian; CGI severity score of 3.6 (0.6) Safety: ERβ was safe and well tolerated with no evidence of QTc prolongation or concerning AEs related to study drug. Target Selectivity: No participants demonstrated suppression in TT or signs of feminization, indicating that LY500307 did not engage ERα and was selective for ERβ. Analysis of brain target engagement and effects on negative symptoms and cognition are ongoing and will be presented at the meeting.

**Conclusions:** LY500307 was a safe, well-tolerated and selective for ERβ at all doses tested. Target engagement and efficacy data will be presented.

**Keywords:** Schizophrenia Novel Treatment, Estrogen Receptor, Cognition

**Disclosure:** Nothing to disclose.

### T186. Reduced Cortical Connectivity of the Anterior Hippocampus in First Episode Psychosis is Remediated by Second Generation Antipsychotic Treatment

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**Background:** Prodromal and first episode psychosis (FEP) stages of schizophrenia have been associated with metabolic and structural alterations in the hippocampus (HPC) that may be a target for early therapeutic intervention. Resting state functional connectivity (rs-fc) based on interregional coherence in 0.01–0.001 Hz fMRI BOLD fluctuations can detect subtle abnormalities and drug associated changes in neuronal wiring and integrity. This method has not yet been applied in FEP patients to characterize hippocampal–brain rs-fc and assess the effects of antipsychotic treatment in a manner that distinguishes between anterior and posterior HPC subregions. This is despite known heterogeneity along the anterior-posterior axis and previous findings implicating the anterior HPC in early SZ. We used a data-driven multivariate analysis to contrast subregional hippocampal–brain rs-fc between FEP subjects and healthy controls (HC); to assess the effects of second-generation antipsychotic (SGA) treatment, and to determine whether baseline rs-fc predicted response to this treatment.

**Methods:** Resting state BOLD fMRI images were acquired from a sample of 61 antipsychotic medication naive FEP patients eight weeks after initiation of second-generation antipsychotic (SGA) treatment, 27 FEP patients were re-imaged and repeat imaging was performed after 8 weeks in 27 age and gender matched HCs. The baseline FEP sample $N = 61$ was split into two groups: completers $N = 27$ and the remainder, $N = 34$ to assess internal reproducibility. Hippocampal-brain rs-fc was analyzed (separately in each FEP group) using masked hippocampal group independent component analysis with dual regression (GICA-DR), yielding multivariate rs-fc for 10 hippocampal independent components (ICs), three in the anterior HPC, one in the mid- and one in the posterior HPC, bilaterally. Group contrasts were calculated with FSL randomise, family wise error corrected, $p < 0.05$. Using a random forest model, 40 features from baseline GICA-DR stage 2 spatial maps were used to predict response to SGA defined based on a median split in reduction in Brief Psychiatric Rating Score.

**Results:** At baseline, FEP subjects had decreased rs-fc relative to HC between the left anteromedial (AM) hippocampal IC and cortical regions including the cingulate cortex and insula, $p < 0.05$, for both FEP groups. Areas of reduced left AM-HPC–brain rs-fc were internally reproducible between groups (Dice coefficient 0.71). Eight weeks of SGA treatment was associated with increased rs-fc in FEP completers, $p < 0.005$. Among completers, a random forest with 4 baseline rs-fc features including left AM-HPC–right

insula rs-fc and left-AM–right superior frontal gyrus rs-fc predicted membership to reduction in BPRS > 35% with an AUC of 0.95.

**Conclusions:** First episode psychosis is associated with decreased rs-fc between the anterior hippocampus and cortical regions previously implicated in schizophrenia. These results support previous studies implicating the anterior HPC in early schizophrenia pathology. Preliminary analysis suggests that with further development GICA-DR derived anterior hippocampal rs-fc may be useful as a biomarker for response to SGA treatment.

**Keywords:** Hippocampus, First Episode Psychosis, Antipsychotic Response, Resting State, Resting State Functional Connectivity

**Disclosure:** Nothing to disclose.

### T187. Opioid Antagonists are Effective Treatments for the Positive and Negative Symptoms of Schizophrenia: A Meta-Analysis

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**Background:** Selective kappa opioid receptor agonists have been shown to produce psychotic symptoms in healthy volunteers, and these symptoms can be blocked by pan-opioid antagonists. Additionally, kappa opioid receptor dysfunction produces dopamine dysfunction in rodent models similar to the changes observed in patients with schizophrenia. Since 1977, when Gunne and colleagues published the first clinical evidence for naloxone's antipsychotic effects in patients with schizophrenia, 47 clinical trials have followed, including three different FDA approved pan-opioid (kappa, mu, and delta) antagonists (naloxone, naltrexone, and nalmefene) and one kappa antagonist and mu partial agonist (buprenorphine). In nearly all of these trials, the opiate antagonist was given as adjunctive treatment in combination with antipsychotics. These trials used various dosages and conditions, and while many showed marked efficacy, some showed mixed results. Since nearly all of these trials were underpowered, we sought to answer the question of whether these pan-opioid antagonists have therapeutic efficacy in patients with schizophrenia by combining the available literature into a meta-analysis.

**Methods:** We performed a search of online databases and identified 47 trials in patients with schizophrenia where opiate antagonists were used to treat the negative or positive symptoms of schizophrenia. These results were further refined with a predetermined set of exclusion criteria to remove studies that did not contain sufficient data for analysis or were of very low quality (such as lack of blinding or placebo control) resulting in 25 blinded placebo-controlled trials in our final analysis. The clinical data and experimental parameters from these papers was then extracted into a master data table utilizing published methods. Improvement in the schizophrenia scales utilized in these trials (BPRS, PANSS, SAPS, SANS, VBS, IMPS and their subscales) were included as endpoints in our analysis. Estimates of Hedge's g were obtained using a standard parametric random effects meta-analytic model (Hedges & Olkin, 1985). In addition, in order to include studies reporting insufficient data to estimate Hedge's g, we also employed a maximum likelihood estimator of the population-level Hedge's g that can include so-called "vote-counting" methods of whether a significant or positive effect was observed (Bushman & Wang, 2009). However, because of concerns that the fixed-effects and asymptotic assumptions of the maximum likelihood estimator were not met by the data, we employed the bias-corrected and accelerated (BCa) bootstrap (Efron & Tibshirani, 1993) to obtain confidence intervals and p-values for this "combination" model, rather than relying on parametric results, which should provide more stringent control of Type I error rates.

**Results:** We found a significant improvement of kappa antagonists on the best available estimate of total symptomatology taken from each study (traditional random effects model: $d = 0.37$; $p = 0.0045$; $k = 21$ studies; bootstrapped combination model: $d = 0.34$; $P < 0.0001$; $k = 25$). We also found a significant improvement on measures of total symptoms (BPRS, PANSS, SAPS, SANS, IMPS, VBS) in the traditional random effects model ($d = 0.23$; $p = 0.0048$; $k = 14$) and bootstrapped combination model ($d = 0.25$; $P < 0.0001$; $k = 22$). We then stratified by positive and negative symptom scales. We found a significant improvement on all positive symptom scales combined in the traditional random effects model ($d = 0.39$; $p = 0.0046$; $k = 13$) and bootstrapped combination model ($d = 0.38$; $P = 0.0027$; $k = 15$). Additionally, for negative symptom scales, we found a significant improvement in negative symptoms in the boot strapped combination model ($d = 0.61$; $P < 0.05$; $k = 6$) and a non-significant trend towards improvement the traditional random effects model ($d = 0.56$; $P = 0.19$; $k = 6$). To further examine the acute antipsychotic effect of these drugs, we examined the positive symptom hallucinations subscales, we found a significant improvement on the bootstrapped combination model ($d = 0.43$; $P < 0.05$; $k = 13$) and a non-significant trend towards improvement on the traditional random effects model ($d = 0.53$; $P = 0.054$; $k = 10$).

**Conclusions:** Our meta-analysis provides the first conclusive data supporting the use of pan-opioid antagonists as adjunctive treatment with antipsychotics for both the positive and negative symptoms of schizophrenia. We propose that the therapeutic mechanism underlying the efficacy of pan-opioid antagonists is dependent on their ability to antagonize the kappa opioid receptor rather than the mu or delta opioid receptor. This suggests acute antipsychotic effect for a class of compounds that are not dopamine receptor type 2 (D2R) antagonists. Our data is especially relevant because these kappa antagonist produced an improvement of symptoms in patients who were already managed with optimal antipsychotic treatment, and may be useful in treatment resistant patients who do not respond to D2 drugs as a possible alternative to clozapine. Finally, our data suggests that kappa antagonists may represent one of the first effective treatments for the negative symptoms of schizophrenia, of which current treatments are only minimally effective.

**Keywords:** Schizophrenia Novel Treatment, Kappa Opioid Receptor Antagonist, Meta-Analysis, Naltrexone, Buprenorphine-Naloxone

**Disclosure:** Serenikey Therapeutics inc, Employee, Stock / Equity, Patent, Board Member

### T188. Double Blind, Two Dose, Cross-Over Clinical Trial of the Positive Allosteric Modulator at the Alpha7 Nicotinic Cholinergic Receptor AVL-3288 in Schizophrenia Patients

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**Background:** Study of the nicotinic alpha-7 acetylcholine receptor (α7nAChR) dates back to observations of the high rate of smoking in schizophrenia, along with the impact of nicotine on auditory P50 sensory gating, a potential biomarker of cognitive deficits in schizophrenia. While a number of direct α7nAChR agonists have been studied in schizophrenia, they have largely failed to separate from placebo in Phase III studies. An ongoing issue with α7nAChR agonist development is that the α7nAChR is quickly desensitized

in the presence of agonists. A possible solution to this is the use of a positive allosteric modulators (PAMs), which are only active in the presence of the endogenous ligand (acetylcholine). This allows for maintenance of the proper temporal pattern of activation and retention of native kinetics and thus are less likely to cause receptor desensitization.

AVL-3288 is a novel, "first in class" selective, type-I, α7nAChR PAM in development for the treatment of patients with schizophrenia accompanied by cognitive dysfunction. Effects of AVL-3288 on the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) and auditory P50 have been observed in healthy, non-smoking subjects in a recently completed Phase 1a single day administration of AVL-3288, including a moderate effect size improvement for the RBANS ($d = 0.49$, Gee et al, 2017). We describe the first multiple dose trial and the first in a patient population.

**Methods:** 24 non-smoking, medicated, outpatients with schizophrenia or schizoaffective disorder and an RBANS > 61 will be enrolled into this Phase 1b, single-center, randomized, double-blind, placebo-controlled, 3-treatment-phase, cross-over study. Each treatment-phase involves 5 consecutive days of the study drug (at either 10 mg or 30 mg) or placebo followed by a 16-day washout period. Each subject will complete each of the three treatment-phases in a double-blind, randomized order.

The principal outcome measure is the RBANS Total Scale Score, with auditory P50 evoked potential suppression the key target engagement biomarker. Secondary outcome measures include task-based fMRI (RISE task), mismatch negativity, the Scale for the Assessment of Negative Symptoms of Schizophrenia [SANS] and the Brief Psychiatric Rating Scale [BPRS].

**Results:** Through July 2018, 21 subjects have been randomized without any clinically significant treatment emergent adverse effects. Baseline RBANS ($82 \pm 17$) and BPRS ($41 \pm 13$) scores were consistent with moderate impairment. The final study visit is scheduled for early October 2018, with the primary outcome results available for presentation at the 2018 ACNP meeting.

**Conclusions:** Results will be presented in contrast to previous direct agonist trials, and both positive or negative results will be informative about the α7nAChR PAM. Successful results will support a Phase II study.

**Keywords:** Alpha-7 Nicotinic Acetylcholine Receptor, Schizophrenia, Cognition

**Disclosure:** Krog & Partners Incorporated, Honoraria, IQVIA, Honoraria, Alphasights, Honoraria, Kinetix Group, Honoraria, Slingshot, Honoraria, Semantics MR LTD, Honoraria, Transperfect, Honoraria, BVF Partners, Honoraria, Taisho, Grant, Lundbeck, Grant, Boehringer Ingelheim, Grant, NeuroRX, Grant, Teva, Grant, Merck, Grant

## T189. Selective Attention in Schizophrenia, Effects of Augmentation of Medical Treatment with Clonidine

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**Background:** Schizophrenia is characterized by reduced abilities in a large number of cognitive domains. This is not only reflected in electrophysiological parameters of selective attention, such as the P3B amplitude, but also in earlier components, such as mismatch negativity, processing negativity and the P200 amplitude. Recently, we reported increased sensory as well as sensorimotor gating following administration of single dosages of clonidine to the treatment of stably medicated patients with schizophrenia. In the current study we investigated if these positive effects of clonidine could also be extrapolated to later electrophysiological components that are usually reduced in these same individuals.

**Methods:** In a double blind, placebo controlled, randomized yet balanced cross-over design 20 male schizophrenia patients on stable medication were assessed in a selective attention task (auditory oddball paradigm) on 5 separate occasions: once after oral administration of placebo and following single doses of 25, 50, 75 and 150 μg of clonidine added to their current medical treatment. Their results were compared with that of an age and gender matched group of healthy controls that was assessed with this same paradigm, yet only once.

**Results:** We found significantly reduced P200 and P3B amplitudes in our patients in the placebo condition, compared to the healthy controls ($0.29 < p < 0.032$; $0.70 <$ Cohen's $d < 0.76$). Clonidine did not affect P200 amplitude. However, clonidine did decrease average P3B amplitude dose-dependently although this did not reach statistical significance ($p = 0.065$, $d = 0.74$), certainly not with the lower dosages. Neither group effects nor effects of clonidine were found on N100, N200, mismatch negativity or processing negativity amplitudes. Interestingly, P200 amplitudes correlated strongly negative with severity of negative and general symptoms of the patients ($-0.688 <$ RS. $< -0.51$; $0.001 < p < 0.025$); no other correlations with severity of symptoms were found.

**Conclusions:** Our results indicate reduced P200 and P3B amplitudes in medicated patients with schizophrenia, which do not ameliorate by augmentation of their medication with clonidine. Interestingly, P200 amplitudes were associated with severity of negative and general symptoms in schizophrenia, which may pave the way for finding better medications for these difficult to treat symptoms. Given that our previous studies showed that even low dosages of clonidine effectively normalized sensory and sensorimotor gating deficits in this same group of patients, our combined previous and current results show promise for - especially low - dosages of clonidine as add-on therapy in the medical treatment of schizophrenia. However, studies on longer term treatment effects of clonidine in schizophrenia are warranted.

**Keywords:** Schizophrenia Novel Treatment, Clonidine, EEG/ERP Electrophysiology

**Disclosure:** Nothing to disclose.

## T190. Monetary Incentives Shape Behavioral and Neural Precision of Spatial Working Memory

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**Background:** Incentive representation confers a key influence on motivated, goal-directed behaviors. Yet, little is known about how incentives influence neurocognitive circuits in humans. Here we studied the effects of cued and non-cued monetary incentive presentation on spatial working memory (WM) performance using functional magnetic resonance imaging (fMRI).

**Methods:** 33 healthy adults ($F = 12$) performed a spatial WM task that incorporated gain and loss monetary incentives. The sWM task incorporated eye tracking and captured continuous responses via a high-precision, scanner-compatible joystick. The possibility for monetary gain or loss was presented either as a cue prior to each spatial WM trial (cued), or as an initial context instruction across a block of WM trials (non-cued). Images were collected using multi-band sequences and parameters consistent

with protocols from the Human Connectome Project (HCP). Preprocessing followed HCP pipelines, and permuted statistics were used for whole-brain analyses.

**Results:** WM accuracy improved when the possibility for monetary gain or loss was presented in a cued manner ($p < 0.001$, both), and in a non-cued manner for the loss condition ($p < 0.01$). The neutral, baseline spatial WM task engaged canonical frontal, parietal, motor and visual areas ($p < 0.05$, whole-brain corrected). We conjuncted this result with a whole-brain corrected map of cued incentive effects, which revealed signal modulation across parieto-occipital, motor, and anterior cingulate cortices during incentive conditions. A further conjunction of this map with a whole-brain corrected map of non-cued incentive effects showed a highly similar but attenuated pattern. We examined the relationship between trial-by-trial sWM precision (calculated as angular deviation) and voxel-wise signal, which revealed a significant relationship between sWM precision and signal change in the inferior parietal sulcus, precentral sulcus and frontal eye fields. Critically, these precision-related signals were further modulated by incentive.

**Conclusions:** Collectively, our results demonstrate that incentives improved sWM performance, and that distinct patterns of cortical modulation reflected the influence of cued and non-cued incentives on sWM. Furthermore, results highlight specific cortical areas where increased signal tracks trial-by-trial sWM precision. This study pinpoints neuro-behavioral incentive-cognition interactions in humans with translational potential to clinical conditions that may affect these computations.

**Keywords:** Visuospatial Working Memory, Reward Processing, Brain Imaging, fMRI, Cortical Circuit Function, Schizophrenia, Depression

**Disclosure:** Task Licensing--one-time royalty, Royalties

### T191. Schizophrenic Psychopathology is Associated With Deficient Midline Frontal Proactive Cognitive Control Mechanisms

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**Background:** Individuals with schizophrenia have difficulty adapting to new environmental contingencies and thus are unusually challenged when navigating an ever-changing world. Experimentally, behavioral inflexibility has been measured using cognitive control tasks. Although there is ample evidence that schizophrenia is associated with deficient cognitive control, the neural mechanisms underlying the deficit have yet to be understood. Specifically, it is unclear whether poor registration of a cue to modify behavior (proactive cognitive control) or execution of the new behavior to a probe (reactive cognitive control) contribute to the deficit in the disorder, and what aberrations in neural processes are associated with these two aspects of cognitive control. Also, given evidence of cognitive control deficits in biological relatives of individuals with schizophrenia, similar aberrations in neural processes may be associated with genetic liability for the disorder. To better understand the neural processes of cognitive control deficits associated with schizophrenia and genetic liability for the disorder we examined electrophysiological responses of individuals enrolled in a family study of schizophrenia while they performed a cue-probe response contingency task. Separate analyses of electrophysiological responses associated with cues and probes allowed differentiation of neural functions related to proactive and reactive cognitive control processes.

**Methods:** Participants consisted of 60 patients with schizophrenia (PSZ), 38 biological relatives of individuals with schizophrenia (RSZ), and 52 healthy control participants (HC). Participants completed diagnostic interviews, symptom rating assessments, measures of schizotypal personality factors, and cognitive testing. Electroencephalography (EEG) data were acquired during cognitive control processes using the Stimulus Response Reversal Task (SRRT) that tapped a prepotency toward a specific behavioral response and equalized the frequency of low and high cognitive control trials. The task structure facilitated distinction between frequency of trial type and level of cognitive control. Neural correlates of cognitive control were examined through analysis of event-related potentials (ERPs) that allowed separation of proactive and reactive cognitive control functions. Participants also completed diagnostic interviews, symptom rating assessments, measures of schizotypal personality factors, and cognitive testing.

**Results:** PSZ performed worse than HCs on the high cognitive control condition. PSZ also performed worse on the high cognitive control than low cognitive control trials. There were no other performance differences across groups or conditions. The cognitive control cue elicited a frontal midline ERP component (N2) that was reduced in both PSZ and RSZ compared to HCs. Both HCs and RSZ groups increased the cue-related N2 response for high cognitive control conditions, but PSZ failed to augment the frontal midline neural response for greater proactive cognitive control demands. Importantly, decremented cue-related N2 amplitudes in PSZ predicted worse performance on the SRRT. Neural responses to probe stimuli were reduced in PSZ and RSZ, but the reductions were independent of the level of cognitive control required. More marked reductions in ERP components during high cognitive control demands predicted more schizotypal characteristics in RSZ. Additional analyses of theta and gamma frequency oscillations will be presented.

**Conclusions:** The present analysis of neural responses during a cue-probe cognitive control task yielded evidence for deficient engagement of proactive cognitive control processes in PSZ and RSZ. The cognitive control deficits were most evident in response to cues for high cognitive control and over midline frontal brain regions. People who carry genetic liability for schizophrenia and report schizophrenia spectrum symptoms tend to exhibit similar abnormalities in frontal neural responses associated with proactive cognitive control. Individuals affected by schizophrenia and schizotypal symptoms appear to exhibit failures in frontal neural mechanisms necessary for representing novel environmental contingencies. Difficulties with new contingencies may in part explain diminished real-world adaptive functioning in people affected by schizophrenic psychopathology.

**Keywords:** Cognitive Control, Schizophrenia, Genetic Liability, Electrophysiology, Midline Theta Frequency

**Disclosure:** Nothing to disclose.

### T192. E/I-Balance and Gamma-Band Oscillations Across the Illness-Stages in Schizophrenia: Insights From Magnetoencephalography

Abstract not included.

### T193. Sleep-Dependent Perceptual Learning in Psychosis

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**Background:** There is increasing evidence that successful learning and memory depends upon sleep and sleep quality [1,2]. As sleep

is impaired in schizophrenia [3], targeting sleep may improve cognition in schizophrenia. About one third of people with schizophrenia report poor sleep quality [3–5]. Patient's perception of their sleep quality is associated with poor quality of life [6]. Polysomnography confirms abnormal sleep in schizophrenia, including increased sleep latency and decreased total sleep time compared with healthy individuals [7,8]. Patients with schizophrenia often have abnormal circadian cycles, and this abnormality predicts poor executive functioning [9]. One possible mechanism for sleep affecting quality of life and cognition in schizophrenia is through impaired sleep-dependent learning.

Studies demonstrating that sleep-dependent learning is impaired in schizophrenia are accumulating. Sleep-dependent improvement on a procedural learning task, a finger tapping sequence task, fails to occur in chronically medicated schizophrenia patients, even though practice-dependent improvement on this task occurred similarly in patients and controls [10]. In a pilot study of 17 patients and 17 matched, healthy controls, overnight visuospatial learning was decreased in patients, and correlated with amount of slow wave sleep and sleep efficiency in patients, though a procedural learning task of figure tracing was not significantly impaired in patients or related to sleep measures [11]. Patients with schizophrenia fail to have improvements in procedural learning after napping, unlike patients with major depression or healthy control participants, even though baseline performance on the task in the three groups was similar [12]. A recent study suggests that some types of sleep-dependent learning may be spared in schizophrenia, namely visuoperceptual learning [13]. This recent study is the only one to our knowledge to examine sleep-dependent consolidation of perceptual learning in schizophrenia. In this study, we examine visuoperceptual learning with another task, visual backward masking.

**Methods:** This is an on-going study recruiting adults ages 18-55 with schizophrenia or schizoaffective disorder and healthy volunteers. Exclusion criteria include poor corrected vision, active substance use disorder, clinical instability (medication changes, hospitalization or housing changes in the last month), neurological illness or sleep disorder. The perceptual task used is computer-administered visual backward masking, as described previously [14]. Participants complete 3 administrations of the task, with 12 h intervening. Participants are randomly assigned to have overnight sleep versus daytime wakefulness in between the first two task administrations.

**Results:** To date, 9 patients and 6 controls have completed the study. Repeated measure anova showed a significant time by diagnosis by schedule interaction ($F = 5.645$, $p = 0.039$), driven by loss of learning overnight for patients and gains during daytime for patients for a visual backward masking task.

**Conclusions:** Given that neuroplasticity impairments in schizophrenia both drive cognitive impairment and pose limitations on cognitive treatment, further understanding of which learning and neuroplastic mechanisms are impaired versus intact can guide treatments to address the cognitive impairments of schizophrenia. From these preliminary results, it seems that patients with schizophrenia lose gains on perceptual learning overnight and do not benefit from sleep-dependent consolidation.

**Keywords:** Schizophrenia and Cognition, Sleep-Dependent Learning, Perceptual Learning

**Disclosure:** Nothing to disclose.

### T194. Predictive Coding During Auditory and Visual Oddball Tasks in Schizophrenia and the Psychosis Risk Syndrome

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**Background:** Amplitude reduction of the P300 event-related potential (ERP) component is among the most replicated biological findings in schizophrenia. Typically, P300 is elicited during an "oddball" task by infrequent target and novel deviant stimuli (P3b and P3a components, respectively) that are randomly interspersed among frequent identical standard stimuli. While deviant stimuli have physical features that explicitly distinguish them from the standard stimuli, the standards can also be distinguished based on their local sequential probabilities within the stimulus series. Therefore, standard stimuli should also elicit a P300 if they are relatively unlikely to occur within local sequences of standards, as the implicit context created by local sequential probabilities can render these stimuli improbable and therefore, deviant. Previously, we showed that healthy individuals generate a P300 (P3a) in response to low probability standards during an auditory oddball task, suggesting that they implicitly recognized the conditional improbability of a standard following a local sequence of multiple repeating standards. However, chronic schizophrenia patients failed to generate this response. The present study examined whether these deficits in P3a associated with standard stimuli are evident early in the course of schizophrenia illness and prior to the onset of psychosis during the psychosis risk syndrome.

**Methods:** ERPs were recorded from 19 young patients with early illness schizophrenia (SZ), 43 individuals meeting psychosis risk syndrome criteria (PRS), and 43 healthy comparison participants (HC) during separate auditory and visual oddball tasks consisting of infrequent target and novel deviant stimuli (20%) imbedded in a series of frequent standard stimuli (80%). Consecutively presented standards following the target or novel stimuli varied in sequential probability from $p = 1.0$ for the 1st standard to $p = .27$ for the 5th consecutive standard. EEG epochs of standard trials were binned according to the number of intervening standards between the eliciting standard and the preceding deviant. The ERP to the 1st standard was subtracted from the ERPs to standard #2, #3, #4, and #5 to extract the P3a. All analyses were based on values extracted from these difference waveforms.

**Results:** A group (SZ, PRS, HC) x standard number (#2, #3, #4, #5) x oddball task modality (auditory, visual) ANOVA revealed a main effect of standard number, $F(3,306) = 49.57$ ($p < .001$), indicating that lower probability standard stimuli elicited a larger P3a across both auditory and visual modalities. However, this standard number effect was qualified by a group x standard number interaction, $F(6,306) = 2.58$ ($p = .026$). Although the standard number effect was evident in all three groups, there was a greater linear increase in P3a amplitudes to lower probability standards in HC ($p < .001$) and PRS ($p < .001$) relative to SZ ($p = .01$). Follow-up Helmert contrasts indicated that, in the HC and PRS groups, P3a amplitudes to lower probability standards were greater than the average amplitudes to higher probability standards (ps < .001), whereas similar effects were not present in SZ. P3a amplitude to each standard was also assessed for the effect of group, and SZ patients had reduced P3a amplitudes to the lowest probability standards (Standard #5) relative to HC ($p = .016$) and PRS ($p = .047$) participants, whereas PRS did not differ from HC ($p = .573$).

**Conclusions:** Results provide further evidence that standard stimuli in an oddball sequence can be rendered implicitly deviant based on their sequential improbability. Furthermore, the previously observed failure of chronic schizophrenia patients to implicitly process local sequential stimulus probabilities was evident in early illness schizophrenia but not during the psychosis risk syndrome, consistent with the emergence of deficient task-based predictive coding with schizophrenia onset.

**Keywords:** Schizophrenia, Clinical High Risk for Psychosis, P300, Event-Related Potentials

**Disclosure:** Nothing to disclose.

### T195. Flexible Adaptation of Frontal and Limbic Networks to Increased Cognitive Demand

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**Background:** While resting state connectivity studies have established canonical maps of cortical and subcortical networks, it remains less clear how these networks adapt or reconfigure in response to cognitive challenges. Indeed, failure of these networks to adapt to increased demand may contribute to cognitive impairment in schizophrenia and other disorders. Cortical-hippocampal circuits are of particular interest in this regard, and in particular, the dynamic crosstalk between prefrontal cortex and functional subdivisions of the hippocampus that occurs in response to increased task demand. Here, we analyzed the functional connectivity between functional subdivisions of the hippocampus and anterior prefrontal cortex (aPFC), a region located at the intersection of the default, limbic, and frontoparietal control networks (FPCN), in a group of healthy adults at rest and during verbal working memory performance.

**Methods:** 182 healthy adult subjects (93 female), age 18-35, completed an fMRI sequence comprising a resting state run immediately followed by a version of the Sternberg Item Recognition Paradigm (SIRP). Working memory load was parametrically modulated through a range from 1 to 7 items. All functional images underwent standard preprocessing (realignment to the first acquired image, registration to a T1 template in the MNI space, smoothing with a 6-mm Gaussian kernel), and connectivity-specific preprocessing [bandpass filtering ( < 0.08 Hz), regression of 6 motion parameters, global signal, and average signal from the ventricles and the white matter]. We computed functional connectivity from two seeds in the right aPFC (one situated within the canonical limbic network, another within the frontoparietal network) to the functional subdivisions of the hippocampus. To do this, we first parcellated the ipsilateral hippocampus based on its resting state functional connectivity profiles using the Yeo et al. 7-network cortical parcellation as reference. Each voxel in hippocampus was assigned to a network based on its strongest correlated cortical network at rest. Then, we computed the average timecourses extracted from these functional subdivisions and correlated them with the timecourses from the two seeds in the aPFC. An ANOVA with within-subject factors Condition (Rest, Task) and aPFC seed (Limbic, FPCN) evaluated task-related differences in cortical-hippocampal connectivity. Finally, we determined the correlation between working memory performance reflected in reaction times and task-induced change in connectivity between aPFC seeds and the hippocampus.

**Results:** Hippocampal parcellation revealed that three main cortical networks (visual, default, and limbic) are represented in the hippocampus, accounting for 94% of the voxels. As subjects transitioned from rest to task, we observed a 10% decrease in mean territory of the default network subdivision and a 9% increase in that of the visual network subdivision. Limbic network territory remained stable regardless of the task condition (2% increase during task). We observed a significant interaction of Condition and aPFC seed [$F(1,724) = 4.31$, $p = 0.038$], reflecting decreased connectivity between the limbic aPFC seed and right anterior (limbic subdivision) hippocampus, and increased connectivity between the FPCN aPFC seed and right anterior hippocampus, when subjects transitioned from rest to task. Finally, behavioral performance measured by the reaction time during the highest working memory load correlated inversely with task-induced change in connectivity between the FPCN aPFC seed and the right anterior hippocampus ($r = -0.22$, $p = 0.0036$).

**Conclusions:** Flexible reconfiguration of prefrontal-hippocampal networks plays an important role in working memory maintenance. Specifically, functional connectivity between right anterior hippocampus and right aPFC, a region located at the intersection of working memory-related networks, shows consistent and behaviorally relevant changes from resting state to working memory performance. Ongoing work is testing for changes in coupling between these two regions in patients with schizophrenia and how it relates to working memory impairment in these patients.

**Keywords:** Task-Based Functional Connectivity, Working Memory, Hippocampus, Anterior Prefrontal Cortex

**Disclosure:** Nothing to disclose.

### T196. Influences of Traumatic Experiences on Risk of Psychosis and Other Major Psychiatric Illnesses

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**Background:** People exposed to traumatic experiences are more than twice as likely to develop psychosis and other commonly comorbid disorders (e.g., substance use disorders, depression, anxiety) throughout their lifetime. While it has been postulated that the linkages between trauma exposure and psychiatric illness are moderated and/or mediated by genomic risk (e.g., diathesis-stress hypothesis), few large, well-powered molecular genetic studies have empirically examined this relationship. Further, few studies have included individuals from racially diverse populations exposed to a variety of traumatic events. In this study, we determined whether we can increase our power to detect etiologically relevant genetic variation in psychosis and related mental disorders by testing for differences (i.e., etiological heterogeneity) by trauma exposure.

**Methods:** We investigated whether polygenic associations with psychotic illness, substance use, depression, and anxiety disorders differ as a function of trauma exposure by analyzing data from the Genomic Psychiatry Cohort (GPC), the largest collection effort among persons of African ancestry to date, with 6,152 schizophrenia cases and 3,918 screened controls among 12,548 individuals, alongside the Grady Trauma Project (GTP) data consisting of over 7,941individuals of African ancestry with extensive assessment of traumatic exposures and psychiatric disorders. Principal components analysis (PCA) was performed with GCTA (v1.2.4; Yang et al. 2011), using a genome-wide genetic relatedness matrix estimated for the GPC datasets and reference samples from the 1000 Genomes Project Phase 3 data based on directly genotyped SNPs. For each individual, we estimated genome-wide average proportions of African, European, etc. ancestry by a linear mixed model (LMM) approach. Using these estimated proportions, we assigned individuals to admixed African ancestry cohorts. Correlations among African ancestry and self-reported "Black/African-American" race is high ($r > 0.8$). Linear mixed models were carried out with the following covariates: age, gender, and ancestral principal components. We examined the effects of polygenic influences on schizophrenia, bipolar disorder, and other severe mental illnesses, derived from recently published GWAS data from the Psychiatric Genomic

Consortium's working groups. In addition, we examined effects as a function of trauma exposure and probable PTSD (i.e., PTSD screening items).

**Results:** Preliminary findings from the GPC indicate that rates of trauma exposure are more prevalent among individuals with psychotic illness as compared with controls. Among people without a diagnosis, 19.2% of the sample reported experiencing a traumatic exposure as compared with 67.7% of those with schizophrenia, 48.3% of those with bipolar, 67.8% of those with a substance use disorder, 60.5% of with depression, and 63.7% of those with anxiety disorders. Self-reported trauma exposure was significantly associated with earlier age-of-onset of schizophrenia among cases with European (Beta = -2.16, 95% CI:[-2.70,-1.64]; P = 3.62e-16) and African ancestry (Beta = -1.89, 95% CI:[-2.45,-1.33]; P = 3.45e-11). Polygenic risk scores constructed from Psychiatric Genomics Consortium (PGC) summary statistics were not significantly different between exposed and unexposed cases with European (P = 0.475) and African (P = 0.279) ancestry. Self-reported exposure to trauma was highly significantly associated with a diagnosis of schizophrenia or schizoaffective disorder in European (OR = 3.24, 95% CI:[3.19 3.30]; P = 8.71e-98) and African ancestry populations (OR = 3.35, 95% CI:[3.29 3.42]; P = 1.51e-69), incorporating self-reported trauma exposure to the polygenic prediction model, and explained an additional 7% and 8.2% of variance (Nagelkerke's R2), respectively.

**Conclusions:** Rates of traumatic exposure are disproportionality greater among individuals with psychiatric illness. Further, polygenic risk for psychiatric disorders is greater among the trauma exposed. These findings provide molecular evidence that trauma exposure exacerbates genetic risk for psychiatric disorders. In addition, these data show that considering trauma exposure in psychiatric genetic studies may increase our ability to detect important genetic influences on psychiatric illness. Ongoing analyses will extend this work in the GTP to determine how polygenic risk for psychosis and trauma come together to increase risk for psychiatric illness in the general population of individuals of African ancestry. This work will further consider the impact of specific types of trauma, age, duration and frequency of trauma exposure to gain further understanding of these findings.

**Keywords:** Trauma Exposure, Polygenic Risk Score, Serious Mental Illness

**Disclosure:** Nothing to disclose.

### T197. Schizophrenia Risk Variants in 6p22.2 Associated With Functional Dysconnectivity

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**Background:** Schizophrenia (SZ) is a psychiatric disorder with estimated heritability of ~80%. Recent genome-wide association studies (GWAS) suggest a polygenic model of SZ. From a neurobiological perspective, SZ patients present altered brain structure and function, among which one important feature is resting-state functional network connectivity (FNC). FNC is affected by genetics; consequently, we examine whether genetic variants contribute to SZ by disrupting the FNC, a key element in pathophysiological pathways from genome to clinical outcomes. In this work, we focused on single nucleotide polymorphisms (SNPs) with top SZ relevance (p < 5.00E-07) in the Psychiatric Genomic Consortium (PGC) GWAS of SZ to study their associations with resting-state FNC in a data driven manner.

**Methods:** We analyzed SNP and resting-state functional magnetic resonance imaging (fMRI) data of 171 healthy controls and 134 patients with SZ recruited from the COBRE and FBIRN projects. The SNP data went through the standard imputation and quality control. After the SNP data were pruned at r2 < 0.9, the threshold of p < 5.00E-07 yielded a total of 1546 SNPs for subsequent analysis. Independent component analysis (ICA) was then applied to the SNP data (305 subjects × 1546 SNPs) to extract 33 components along with their associated loading vectors. Each ICA component captures a cluster of variables covarying across subjects and the associated loading vector reflects the shared covariation pattern. In parallel, the images were preprocessed with a standard SPM8 pipeline of realignment, normalization, and 6 mm smoothing. Group ICA (GICA) was conducted to extract 75 components, 50 of which were characterized as meaningful networks and their time courses were further detrended, motion corrected, despiked and bandpass filtered at 0.01-0.08 Hz. An FNC matrix was constructed for each subject based on the correlations among time courses of these 50 components, leading to 1225 connectivity features. ICA was then applied to the FNC data (305 subjects × 1225 features) to extract 16 components, which captured clusters of network connectivities covarying across subjects. We then identified the FNC components that showed significant case control differences (passing Bonferroni correction for 16 components) with two-sample t-test controlling for age, gender, and site. Finally, we examined if any significant associations (passing Bonferroni correction for all the SNP-FNC pairs) existed between the group-discriminating FNC components and the 33 SNP components with partial correlation controlled for age, gender, diagnosis, site and the top 3 principal components of the genomic SNP data.

The SNP-FNC association identified in the COBRE + FBIRN data was further assessed for replicability in the independent HCP data which consisted of 356 unrelated healthy individuals. The HCP SNP data were downloaded as imputed and went through the same quality control as the COBRE + FBIRN data. The HCP fMRI data were downloaded as preprocessed by the consortium's protocol. For the purpose of replication, we assumed that the multivariate ICA and GICA components should generalize to new data. Consequently, for the SNP modality, the COBRE + FBIRN SNP components were projected to the HCP data to yield the HCP SNP loadings. For the FNC modality, first the subject level components and time courses were back-reconstructed from the COBRE + FBIRN GICA components using the group information guided ICA approach, such that the resulting FNC was completely aligned with that of COBRE + FBIRN. Then the COBRE + FBIRN FNC components were projected to the HCP's FNC matrix to yield the HCP FNC loadings. We then examined whether a significant association (p < 0.05) still existed between the projected SNP and FNC loading vectors in HCP.

**Results:** A total of 4 FNC components were identified to present significant case-control differences. Among all the combinations of 4 FNC components and 33 SNP components, one SNP-FNC pair showed a significant association (r = 0.20, p = 3.52E-04). The associated FNC component showed significantly higher loadings in controls than patients (p = 6.86E-07) and overall highlighted more positive/less negative default-mode network (DMN) connectivities in patients. The SNP component did not show a significant group difference. Nevertheless, the inferred risk alleles for SZ-related functional dysconnectivity from our SNP-FNC association were all consistent with the inferred risk alleles for SZ from the PGC GWAS results. The highlighted SNPs resided in 6p22.2 and the annotated genes included HFE, SCGN, and three phosphate transport protein genes SLC17A1, SLC17A3 and SLC17A4. Finally, when projected to HCP, the resulting SNP-FNC association remained significant (r = 0.13, p = 1.40E-02).

**Conclusions:** The identified SNP-FNC association suggests a possibility of shared genetic risk between functional

dysconnectivity and SZ. The HFE gene affects myelin-related molecular systems. The SCGN gene is reported to encode neuron specific Ca2 + -binding protein. The observed DMN hyperconnectivity in SZ is also consistent with the existing literature. Although the functional annotations await further elucidation, collectively the robust imaging genetic association suggests the SZ risk SNPs in 6p22.2 relate to DMN hyperconnectivity, demonstrating a possible pathway of FNC mediating genetic influences on SZ.

**Keywords:** Schizophrenia, SNP, Resting State Functional Connectivity, Default Mode Network (DMN)

**Disclosure:** Nothing to disclose.

### T198. Circuit Control of Sub-Chronic Ketamine-Induced Striatal Dopaminergic Overactivity: A Combined Chemogenetics/PET Study

Abstract not included.

### T199. Genome-Wide Association Analysis Identifies Genes Associated With Dentate Gyrus Volume in Schizophrenia

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**Background:** Schizophrenia is associated with robust total hippocampal volume deficits but hippocampal subregion volume deficits, their associations with cognition, and contributing genes remain to be determined.

**Methods:** Hippocampal formation subregion volumes were obtained using FreeSurfer 6.0 from individuals with schizophrenia (n = 176, mean age ± SD = 39.0 ± 11.5, 132 males) and healthy volunteers (n = 173, mean age ± SD = 37.6 ± 11.3, 123 males) with similar mean age, gender, handedness, and race distributions. Relationships between the hippocampal formation subregion volume that showed the largest between group difference, neuropsychological performance, and single-nucleotide polymorphisms were assessed.

**Results:** This study found a significant group by region interaction on hippocampal formation subregion volumes. Compared to healthy volunteers, individuals with schizophrenia had significantly smaller hippocampal formation subregion volumes in the dentate gyrus, Cornu Ammonis 4, molecular layer of the hippocampus, hippocampal tail, and Cornu Ammonis 1; dentate gyrus and Cornu Ammonis 4 volumes remained smaller when statistically controlling for mean hippocampal volume. Among subregions, dentate gyrus volume showed the largest between group difference (Cohen's d = -0.57) and was significantly correlated with working memory performance in schizophrenia (r159 = 0.25, 95% CI: 0.1-0.39, p = 0.001). Genome-wide association analysis with dentate gyrus volume identified rs56055643 at $p < 5 \times 10-8$ (Beta = 10.8, 95% CI: 7.0-14.5) on chromosome 3 in high linkage disequilibrium with MOBP. Gene mapping and gene-based analyses identified associations between MOBP, SLC25A38, and RPSA and dentate gyrus volume.

**Conclusions:** This study suggests that dentate gyrus dysfunction is fundamentally involved in the pathophysiology of schizophrenia, that it may contribute to working memory abnormalities in schizophrenia, and that underlying biological mechanisms may involve genetic contributions from MOBP, SLC25A38, and RPSA.

**Keywords:** Schizophrenia, Hippocampus, Dentate Gyrus, Volume, GWAS

**Disclosure:** Nothing to disclose.

### T200. α7-NAChR: Brain Imaging of [18 F] ASEM With Reproducibility, Specific Binding, and Changes in Schizophrenia

Abstract not included.

### T201. Abnormal Ventral Striatum Activation During Effort Discounting Correlates With Clinical Amotivation Severity in Schizophrenia

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**Background:** Motivational deficits play a central role in disability due to negative symptoms of schizophrenia and constitute a major unmet therapeutic need. However, the pathophysiology of amotivation remains largely unknown, and progress will depend on integrating clinical assessment with quantitative behavioral and imaging phenotypes. Here we applied an fMRI Effort Discounting Task (EDT) that quantifies motivation using a neuroeconomic decision-making approach, capturing the degree to which effort requirements produce reductions in (discount) the subjective value of monetary reward. We hypothesized that brain motivation circuitry including ventral striatum (VS) and anterior cingulate (AC) would encode subjective value, integrating reward and effort costs. We expected reduced task effort and impaired subjective value encoding in schizophrenia (SZ), correlating with dimensional severity of clinical amotivation.

**Methods:** A sample of 22 patients with SZ (stable/medicated) and 23 group-matched controls (CT) performed EDT during fMRI (3 T BOLD). In each of 200 trials, subjects chose between higher-effort/higher-reward (HARD) and lower-effort/lower-reward options (EASY). The required effort involved repetition of easy but attention-requiring trials (choosing which of 2 numbers is larger). The reward and effort magnitudes for the HARD option were parametrically and independently varied across trials. Behavioral analysis of EDT applied a quantitative neuroeconomic model capturing how the subjective value of a decision to perform an effortful task incorporates the tradeoff between monetary reward and effort costs, using the equation SV = A-B*E. This equation describes how the subjective value (SV) of a particular monetary reward amount (A) is reduced or "discounted" as the effort cost (E) needed to obtain it increases. Higher values of the estimated free parameter B indicate a stronger negative impact of effort on subjective value, and hence lower motivation. fMRI analysis focused on VS, AC, and associated valuation and decision-making circuitry using both ROI and voxelwise analyses. The primary clinical outcome was amotivation rated via the Clinical Assessment Interview for Negative Symptoms (CAINS).

**Results:** As expected, EDT choices were accurately predicted by the linear model outlined above, and poorly predicted by the hyperbolic model used in nearly all temporal discounting studies and a significant proportion of prior effort discounting work. Motivation indexed by the EDT ranged widely in both groups without categorical group differences and showed trend correlations with CAINS amotivation (r's ~0.3) in both CT and SZ. Model fit and reaction times did not relate to diagnosis or CAINS

amotivation. VS and AC, as well as a broader cortico-limbic network, were activated during the decision task and this activation correlated positively with subjective value. The correlation with SV reflected both a strong inverse relationship with parametric effort variation across trials, and a weaker positive relationship with parametric variation in monetary reward. The negative relationship of effort magnitude to fMRI activation in this valuation network was stronger in those with lower EDT motivation across all participants (VS $r = 0.40$, AC $r = 0.54$), with similar effects in both SZ and CT. Activation in the nucleus accumbens (NAc, ventromedial VS) was selectively related to the subjective value of the chosen option (whole brain peak Z 4.08 at MNI coordinate -8, 16, -2). NAc activation to task decisions (independent of parametric variation in reward and effort) was selectively reduced in SZ, and greater NAc reductions correlated with more severe CAINS amotivation in SZ as well as across all participants (small-volume cluster-corrected p's < 0.05). Surprisingly, the chosen value fMRI parameters in NAc actually correlated positively with CAINS amotivation severity in SZ.

**Conclusions:** Findings demonstrate that ventral striatum and anterior cingulate, together with a broader cortico-limbic network, integrate effort costs and reward benefits into subjective values that drive decision-making, consistent with prior work in healthy individuals as well as animal models. The fMRI abnormalities we observed in schizophrenia were selective for ventral striatum, and within this region correlated with severity of clinical amotivation. This adds to growing evidence that VS is a critical region for motivation impairment in schizophrenia as well as other psychiatric conditions. Neuroeconomic approaches add interpretive specificity as well as complexity to this central conclusion. The reduction of the VS response to the overall task, coupled with apparently increased sensitivity of VS to variation in the value of the chosen option, suggests that while overall valuation of effortful decisions is lower in association with amotivation in SZ, modulation within this lower value range remains intact or even heightened.

**Keywords:** Functional MRI (fMRI), Motivation, Effort Discounting, Schizophrenia

**Disclosure:** Nothing to disclose.

### T202. Mu-Opioid Receptor Availability and its Relation to Reward Function in Schizophrenia Patients: A Multimodal Brain Imaging Study

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**Background:** Negative symptoms in schizophrenia are conceptualised as a constellation of symptoms such as blunted affect, poverty of speech, amotivation, anhedonia and associality. Although negative symptoms are present in approximately 60% of the patients and are associated with poor functional outcome, there are no effective treatments. While a substantial number of studies have examined the neurobiology of positive symptoms, the molecular mechanisms underlying negative symptoms are less studied. Dopaminergic dysregulation is a well-established finding in schizophrenia and there is increased interest to explore presynaptic modulators of dopamine neurotransmission. Preclinical-studies indicate that Mu-opioid receptors (MOR), presynaptically modulate dopamine neurotransmission. Specifically, stimulation of MOR leads to an activation of dopamine neuron via GABA mediated dis-inhibition. Positron emission tomorgraphy (PET) studies have shown that dopamine synthesis capacity in striatum is positively correlated with MOR availability and stimulation of MOR using remifentanil leads to dopamine release. Further, pre-clinical evidence suggests that opioid neurotransmission in the striatum is linked to hedonic and reward function, and MOR knock out animal models display anhedonic behaviour. However, the MOR availability in-vivo and its relation to negative and reward function in schizophrenia has not been studied. Using a MOR specific ligand [11 C]-carfentanil, we report the first in vivo study of MOR availability in patients with schizophrenia.

**Methods:** We compared MOR availability in 20 patients with moderate negative symptoms and 20 age and sex matched healthy controls using [11 C]-carfentanil PET. All patients met DSM-V criteria for schizophrenia with at least one negative symptom with a score $>/=4$ on the positive and negative symptom scale (PANSS) negative symptom sub-scale OR two or more negative symptoms with a score $>/=3$ on the PANSS negative symptom sub-scale. All patients were on antipsychotics for at least four weeks prior to the PET scan. Regional estimates of the binding potential (BPND) of [11 C]-carfentanil were measured using simplified reference tissue model with occipital cortex as reference tissue. Primary region of interest was defined as the striatum as it is rich in MOR and its dysfunction is reported in schizophrenia. Secondary analysis evaluated group effects on MOR availability in amygdala, frontal lobe, temporal lobe, parietal lobe, thalamus, cingulate cortex and hippocampus. Functional Magnetic Resonance Imaging (fMRI) of brain responses during a monetary incentive delay task also measured the neural response to reward anticipation. In addition, social anhedonia rating scale, temporal experience of pleasure scale and behavioural inhibition system/behavioural activation system rating scales were administered to the participants to assess the anhedonia.

**Results:** There were no significant differences in the age, gender and radio-activity administered between groups. There was an outlier in patient group with high BPND (Grubbs test $p < 0.05$) and these data were excluded from further analysis. There was a significantly lower striatal MOR availability in the striatum of patients with schizophrenia (patients vs controls (mean ± SD): $1.54 \pm 0.26$ vs $1.7 \pm 0.22$, Cohen's $d = 0.7$, $p = 0.037$). Secondary analysis revealed significant group effects on BPND measures ($p < 0.001$). There was also hypoactivation of ventral striatum in schizophrenia patients, however there was no correlation between MOR availability and striatal activation ($p > 0.05$). There was no correlation between negative symptom, anhedonia measures and MOR availability in striatum.

**Conclusions:** Schizophrenia patients with moderate negative symptom have reduced MOR availability in the striatum. This finding may have implication in understanding the neurobiology of negative symptoms.

**Keywords:** Molecular Imaging, Mu-Opioid Receptors, Schizoprenia

**Disclosure:** Nothing to disclose.

### T203. Low Availability of the Alpha7 Nicotinic Acetylcholine Receptor in Recent Onset of Psychosis: A Pilot Study With [18 F]ASEM PET

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**Background:** Low hippocampal availability of the α7 nicotinic acetylcholine receptor (α7-nAChR) in non-affective psychotic disorders (NP) such as schizophrenia has been suggested through

study of postmortem tissue. If validated, the deficient cholinergic signaling through the α7-nAChR in psychosis may have a mechanistic link to altered hippocampal functional activity contributing to cognitive and psychotic phenomena. Here we used [18 F]ASEM with positron emission tomography (PET) to test for hypothesized low in vivo availability of the hippocampal α7-nAChR in recent onset psychosis, including NP or affective psychotic disorder (AP), compared to healthy controls.

**Methods:** This prospective study was approved by a Johns Hopkins Institutional Review Board. Each non-smoker participant provided written informed consent. Individuals with recent-onset (within five-years) psychosis were included if they met DSM-IV criteria for: 1) schizophrenia or schizoaffective disorder (grouped NP) or 2) bipolar I disorder (referred to as AP). Limited medication use (lithium or antipsychotic monotherapy) was allowed. Eleven patients and five new healthy controls completed [18 F]ASEM PET, and we pooled their data with those of all ten healthy individuals < 50-years-old from our published study of the α7-nAChR in healthy aging. [18 F]ASEM kinetics were modeled using Logan graphical analysis with a metabolite-corrected arterial input function from 90 min dynamic data. Hippocampal total distribution volume (VT) values were derived from images after partial volume correction (PVC). Group differences in VT were tested using analysis of variance and using analyses of covariance to control for potential confounding effects of age, sex, race, or body mass index (BMI).

**Results:** Among individuals with recent-onset psychosis, five had NP [schizophrenia (N = 3), schizoaffective disorder (N = 2)] and six had AP. There were significant group differences [using three groups (Controls, AP, NP) or two groups (Controls, AP + NP)] on hippocampal VT (Ps ≤ 0.001), even after adjusting for age (each P = 0.001). Individuals with recent onset psychosis (AP + NP) had lower VT (15.97 ± 2.50) than healthy controls (19.55 ± 2.49, P = 0.001), though VT in the AP group alone (17.57 ± 2.24) did not differ from healthy controls. VT was lower in individuals with NP (14.05 ± 0.89) compared to healthy controls (P < 0.001) or compared to those with AP (P = 0.04) and remained lower in those with NP compared to healthy controls after adjusting for each covariate separately (Ps ≤ 0.002). Controlling for BMI or race did not change the lower VT in individuals with NP compared to AP (Ps = 0.01), but significance was lost after adjusting for age. Among patients (AP + NP), higher VT was associated with better processing speed and verbal memory after adjusting for age. VT estimates from images without PVC did not change these results and produced parametric images that support group differences outside hippocampus.

**Conclusions:** These results suggest that low availability of the α7-nAChR may be linked to recent onset of psychosis. Further study is needed to assess its clinical relationship to neuropsychiatric symptoms.

**Keywords:** [18 F]ASEM, Recent Onset Psychosis, Alpha7 Nicotinic Acetylcholine Receptor, Positron Emission Tomography

**Disclosure:** Nothing to disclose.

### T204. Differentiated Neural Connectivity Signatures of Active Hallucinations and Delusions in the BSNIP1 Sample

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**Background:** Identifying the unique neural system underpinnings of hallucinations and delusions could be a useful way to parse the heterogeneity of psychotic disorders. Such findings would explain why the typical approach of collapsing across these symptoms has not been elucidating. Neurocognitive hypotheses about each symptom offer models to test. Delusions have been posited to follow from problems with a process called prediction error, normal brain signals indicating the need to update incorrect beliefs when presented disconfirming information. fMRI studies have found delusional beliefs correlate with activation in frontal and subcortical brain regions during prediction error. Hallucinations in psychotic disorders are most commonly auditory-verbal, hypothesized to involve language systems. We aimed to test whether alterations in resting state connectivity to these regions (delusion-associated prediction error [D-PE] regions and language regions) map to respective current symptom severity in the Bipolar and Schizophrenia Network on Intermediate Phenotypes (BSNIP) dataset, a multi-site cross-sectional cross-diagnostic study. BSNIP has proposed three subgroups (Biotypes 1-3) based on neurocognitive biomarkers (not inclusive of resting state fMRI data). We assessed for connectivity-symptom correlates across the patient sample and within both traditional diagnostic and Biotype subgroups.

**Methods:** Seeds for creating D-PE connectivity maps were spheres centered on peak voxels reported in prior task-based prediction error fMRI literature (r dorsolateral prefrontal cortex [rDLPFC], r ventrolateral prefrontal cortex [rVLPFC], r caudate, and l midbrain). Language seeds were generated from the Harvard-Oxford atlas (Broca's, Wernicke's, primary, and secondary auditory cortices). Whole brain resting state connectivity maps were created for each seed for 391 male and female psychosis subjects whose resting state data passed strict quality control assessments (e.g., motion < 2 mm). Univariate regression analysis controlling for age, sex, and site was conducted for each set of connectivity maps to determine association with PANSS items P1 and P3, characterizing current severity of delusions and hallucinations, respectively.

**Results:** Clusters of voxels were identified as significantly associated with P1 or P3 using at p < .05 familywise error correction (including p < .001 individual voxel cutoff). In the full patient sample, delusion severity was associated with decreased connectivity between rVLPFC and lingual gyrus, and neither D-PE nor language connectivity maps associated with hallucination severity. D-PE CONNECTIVITY: For schizophrenia, delusion severity associated with decreased connectivity between rPFC and r hippocampus, and increased connectivity for l midbrain and caudate; hallucination severity associated with increased connectivity of l midbrain to middle frontal gyrus. For schizoaffective, delusion severity associated with lower connectivity between l midbrain and temporal lobe, and hallucination severity did not associate with D-PE connectivity. For bipolar, delusion severity associated with increased connectivity of l midbrain to pons and decreased connectivity of r caudate to r parahippocampus, while hallucination severity associated with decreased connectivity between rPFC and left middle temporal gyrus. LANGUAGE CONNECTIVITY: For schizophrenia, hallucination severity associated with greater connectivity between Broca's area and caudate, and between the receptive auditory cortex seeds and precuneus, basal ganglia, and occipital regions; delusion severity did not show associations with language region connectivity. Schizoaffective patients did not show hallucination or delusion severity correlates with the language area connectivity maps. Bipolar subjects' hallucination severity associated with reduced connectivity between secondary auditory and Wernicke's area to portions of bilateral cerebellum. For the Biotype subgroups, only Biotype 1 showed a delusion severity association (greater connectivity between rVLPFC and anterior cingulate). Biotype 1 showed greater hallucination severity associated with greater connectivity between secondary auditory cortex and occipital lobe. Biotype 2 showed greater hallucination severity associated with increased connectivity between secondary auditory cortex and closely neighboring temporal and parietal cortex. Biotype

3 showed greater hallucination severity associated with increased connectivity between secondary auditory cortex and superior and middle frontal gyrus.

**Conclusions:** Our findings suggest that across the major psychotic disorders, there is little common pathophysiology in resting state fMRI data processed to assess neurocognitively-based hypotheses of altered neural systems underlying delusions or hallucinations. However, there are patterns of altered connectivity identifiable within both diagnostic subgroups and within experimentally-derived biomarker-based subgroups. There was no overlap in any subgroup for locations of altered connectivity associating with delusions and hallucinations, validating the proposal that these positive psychosis symptoms have separate, identifiable neural system underpinnings.

**Keywords:** Bipolar Disorder, Resting State Functional Connectivity, Hallucinations, Delusions, Cortical Circuit Function, Schizophrenia

**Disclosure:** Nothing to disclose.

### T205. Elevated Glutamate Levels in the Superior Temporal Gyrus in Chronic Schizophrenia

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**Background:** Abnormalities of glutamatergic and GABAergic neurotransmission characterize schizophrenia (SZ), a serious mental disorder affecting around 1% of the population worldwide. Glutamate and GABA levels, measurable non-invasively in vivo through magnetic resonance spectroscopy (MRS) have been well characterized in the prefrontal cortex and anterior cingulate of SZ patients. We hypothesized that levels of the two metabolites will be abnormal in the superior temporal gyrus, a brain region that is implicated in SZ and involved in several cognitive operations found abnormal in SZ including auditory, language and social cognition processing.

**Methods:** Fourteen subjects with SZ and 14 healthy controls (HC) matched for age and gender were included in the study. Measures included: GABA and Glutamate levels assessed during resting state MRS in the left and right STG.

Glutamate and GLX (Glutamate + Glutamine) levels were acquired using PRESS pulse sequences and GABA levels were acquired using MEGAPRESS pulse sequences. Data were normalized for gray and white matter and cerebrospinal fluid content of each subject in each voxel. GLX, glutamate and GABA were analyzed using a repeated measures ANOVA model, with Diagnosis (schizophrenia versus control) as a between-subjects factor and hemisphere (left STG and right STG) as a within-subjects factor. Age of subjects added as a covariate, did not contribute to the model and thus was not included in the final analyses. Electrophysiological data, including oddball and novel P300, and mismatch negativity were also acquired. Correlational analyses were conducted between glutamate and ERP components.

**Results:** Gray and white matter volumetric measures in the left and right STG did not show group differences.

GLX, a measure combining glutamate and glutamine showed a trend level significant effect of Diagnosis ($F = 4.0$, $p = 0.056$; $d = 1.13$) with no main effects or interactions for Hemisphere indicating an increase of GLX in the STG in SZ compared to HC. There was a main effect of Diagnosis for glutamate with a significant group difference in the STG with no laterality effects ($F = 5.2$; $p = 0.031$; $d = 0.91$), indicating increased glutamate levels in the patient group. No significant main effect of Diagnosis or any other significant interactions were found for GABA ($F = 0.35$; $p = 0.32$]. Auditory P300 oddball amplitude was reduced in patients relative to controls ($p = 0.001$; $d = 1.5$) Similarly, P300 novel amplitude indexing novelty processing was reduced in the patient relative to the control group ($p = 0.001$; $d = 1.44$). We have also found less negative N1 (analyzed from the P300 oddball, frequent waveform) in the patient group ($p = 0.007$; $d = 1.44$) and reduced P2 at trend level ($p = 0.056$; $d = 0.76$). MMN frequency but not MMN duration was found less negative in the patient group ($p = 0.005$, $d = 1.18$) relative to the HC group. Glutamate levels in the STG were associated with novel P300 and frequency MMN amplitudes. Novel P300 amplitude at FZ-CZ showed a negative correlation with right STG glutamate levels (z scores) in HC ($p = 0.015$) and a positive correlation for the same voxel in the CSZ group ($p = 0.05$). Similarly, frequency MMN amplitude was found to be positively correlated with left STG glutamate levels (z scores) in the CSZ group ($p = 0.01$). In the HC group, the negative relationship between frequency MMN amplitude and left STG glutamate levels was observed but it did not reach a statistical significance.

**Conclusions:** We report here a novel finding of significant increase of glutamate and GLX in the STG of chronic SZ patients. The lack of significant group differences in the STG volume indicates that the observed glutamatergic group differences are not related to the gross loss of volume of the STG voxel. Correlations between electrophysiological measures and glutamate suggest an abnormal relationship between glutamate levels and both auditory pre-attentive and salience processing in schizophrenia.

**Keywords:** Glutamate GABA, Schizophrenia, Auditory Deficits in Schizophrenia, P300, MR Spectroscopy

**Disclosure:** Nothing to disclose.

### T206. Single Nucleus RNA Sequencing on Postmortem Human Brain Classifies Neuronal and Non-Neuronal Cell Types

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**Background:** Recent single cell sequencing studies have made advances in characterizing cellular expression in mice, but isolating intact cells is not feasible from frozen postmortem human tissue. Single nucleus RNA sequencing is a new technology that can be applied to frozen tissue providing transcriptional information at the single cell resolution. In the present study, we investigate the feasibility of this technology using postmortem human brain.

**Methods:** Samples of the subgenual anterior cingulate cortex (sgACC) of two non-psychiatric donors and two patients with schizophrenia were obtained from the Human Brain Collection Core, NIMH. A total of 10,925 nuclei were isolated and Chromium Single Cell 3′ technology from 10X Genomics was used to sequence the nuclear RNA. Data filtering, processing and analysis were performed with Cellranger and Seurat.

**Results:** 6,099 nuclei formed 14 clusters of identifiable cell types. Specifically, 6 excitatory neuronal clusters expressing known markers, SLC17A7 and SATB2, and 3 inhibitory neuronal clusters expressing GAD1 were identified. Astrocytes, oligodendrocytes, oligodendrocyte precursor cells, and microglia were identified by SLC1A2, PLP1, PDGFRA, and CD74, respectively. Inhibitory neurons could be further clustered into distinct known

subtypes (SST, VIP, CCK), however no cluster expressing parvalbumin was found.

**Conclusions:** The non-neuronal clusters corresponded well with a recent study of postmortem human prefrontal cortex and hippocampus, while excitatory clusters showed less overlap, possibly due to differences between cortical regions. These differences in clusters of excitatory neurons have been confirmed in recent reports sampling different cortical areas in mouse and humans. The differences in expression of classic inhibitory markers between subgenual anterior cingulate and dorsolateral cortical areas is also supported by the Allen Human Brain atlas. Future work will aim at further characterization of regional differences in the presence of neuronal subtypes and of cell-specific differential expression in psychiatric disorders vs. controls.

**Keywords:** RNA Sequencing, Single-Nucleus, Subgenual Cingulate Cortex, Post-Mortem

**Disclosure:** Nothing to disclose.

## T207. In Situ Visualization and Quantification of Transcripts Associated With Risk for Psychiatric Disease in Postmortem Human Brain Tissue

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**Background:** Genome-wide association studies (GWAS) have identified many single nucleotide polymorphisms (SNPs) associated with risk for psychiatric illness. These genetic associations tend not to implicate coding regions that change amino acid content of proteins, but rather regulatory regions that impact gene expression and/or alternative splicing. Integrating risk-associated SNPs with functional genomics data, such as bulk RNA-sequencing data, has enabled identification of expression quantitative trait loci (eQTLs), or putative functional variants that influence gene expression impacting risk for psychiatric illness.

**Methods:** To classify eQTL associations in distinct cell types in the intact human brain, we developed methods to identify and quantify cell types harboring risk transcripts across corticolimbic regions in postmortem human tissue. We utilized RNAscope, a novel multiplex single-molecule fluorescent in situ hybridization technology, to localize selected genes associated with risk for psychiatric illness to inhibitory neurons, excitatory neurons, astrocytes, or oligodentrocytes in dorsolateral prefrontal cortex. In parallel, we utilized BASEscope, a cutting-edge in situ hybridization technology that can detect single exon junctions, to localize specific splice variants associated with risk for psychiatric illness to the same cell types.

**Results:** Here we report the development of methods for fluorescent in situ hybridization, multispectral confocal microscopy, and automated image quantification for evaluating the expression of genes and splice variants associated with risk for psychiatric disease in whole cell populations in the intact postmortem human brain. We demonstrate optimized conditions for tissue preparation and probe hybridization using RNAscope and BASEscope technologies. We validate multispectral confocal imaging parameters to 1) identify 4 gene targets and a nuclear marker simultaneously and 2) generate parameters for masking background autofluorescence from lipofuscin. Finally, we develop new tools in Matlab for automated image analysis, including nuclear segmentation and dot detection, to quantify expression of risk genes and transcripts in specific cell types in intact brain tissue.

**Conclusions:** We report and validate techniques for characterizing the cellular identity of eQTL associations in postmortem human tissue in situ. We gain improved resolution of anatomical regions and cell types harboring transcripts associated with disease risk and state and provide a framework for adding biological insights into computational associations in human brain tissue.

**Keywords:** eQTL, Postmortem Human Brain, RNAscope Fluorescence in Situ Hybridization

**Disclosure:** Nothing to disclose.

## T208. Analysis of Risk Factor Domains in Psychosis Patient Electronic Health Records

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**Background:** Readmissions are disruptive for patients and families and a key driver of rising healthcare costs. Reducing readmission risk is therefore a major unmet need of psychiatric care. Developing clinically-informed machine learning tools to enable accurate assessment of risk factors associated with readmission offers opportunities to inform the selection of treatment interventions and implement appropriate preventive measures. In psychiatry, traditional strategies to evaluate readmission risk rely on clinical observation and manual retrospective chart reviews. This approach, although benefitting from clinical expertise, doesn't scale well for large data sets because it is effort intensive and lacks automation. Longitudinal electronic health record (EHR) data, which contain detailed information about a patient's illness history and symptom characteristics, and treatment plans, provide better opportunities to assess readmission risk. However, the use of natural language processing (NLP) in extracting readmission risk factors from clinical narratives in psychiatric patient EHR data presents special challenges. The vocabulary used in psychiatric clinical narratives is highly varied and context-sensitive, the narrative structure varies considerably in how the same symptom (e.g. hallucinations) is described, and symptom presentation of a patient can change considerably over time, leading to various diagnoses and comorbidities. As a result, the lexicon of words and phrases used in EHRs differs not only across diagnoses but also across patients and time. Taken together, these factors make finding key risk factor domains a difficult task that cannot be accomplished by simple text-mining techniques. In the present study, we evaluated multiple approaches to automatically identify which risk factor domains are associated with which paragraphs in psychotic patient EHRs.

**Methods:** The target data set consisted of a corpus of discharge summaries, admission notes, individual encounter notes, and other clinical notes from 220 first episode psychosis patients. Approximately 240,000 total EHR paragraphs spanning from 2011 to 2014 were extracted. We focused on seven risk factor domains – Appearance, Mood, Interpersonal, Occupation, Thought Content, Thought Process, and Substance Use – identified on the basis of being clinically relevant, consistent with literature, replicable across data sets, explainable, and implementable in NLP algorithms.

To train our readmission risk factor topic extraction model, we initially used 100,000 comparable EHR paragraphs from 30,000 psychiatric patients in the Research Patient Data Registry (RPDR), a centralized data repository of clinical data from a large New England health system. These paragraphs were converted to a matrix of TF-IDF vectors, where each word in the paragraph is assigned a weight that corresponds to how informative the word

is in uniquely identifying a particular domain. We then used the resultant matrix to train a three-layer radial basis function neural network. We also trained a different radial basis function network using bag-of-words vectors (simple word counts).

To further improve the accuracy of our model, we incorporated domain-relevant multiword expressions (MWEs) (e.g., 'panic attack') and employed a novel iterative bootstrapping approach to extract additional training paragraphs (~200,000) from RPDR.

Finally, we undertook an annotation task completed by three licensed clinicians who annotated over 1,600 EHR paragraphs for risk factor domains to create a 'gold standard' test set, which we used to evaluate the performance of our models. We received IRB approval to release the corpus to the community for future use by other research groups.

**Results:** On the annotation task, overall inter-annotator agreement was good (Fleiss's Kappa = 0.575), which lies on the boundary between 'Moderate' and 'Substantial'. On classifying the paragraphs in our gold standard, our classifier showed consistency between precision and recall, and consistency in performance between classifying all risk factor domains in a given paragraph versus classifying only the most prevalent domain.

We trained a baseline model that did not include MWEs or bootstrapping training data. This basic model achieved an overall F1 score of 0.57. MWEs significantly improved our model performance with an overall F1 score of 0.68. We observed the highest per-domain F1 scores on Substance and Thought Content (F1 ~ 0.77) and the lowest on Interpersonal and Mood (F1 ~ 0.51).

Compared to the traditional bag-of-words vectorization procedure, we found that TF-IDF vectorization reduces noise and increases model specificity. Results of our neural network model trained using TF-IDF vectorization were 15% better than a model trained using bag-of-words vectors.

Extracting additional training data from RPDR using our novel bootstrapping procedure resulted in further improvement in model performance, achieving an F1 score of 0.72.

**Conclusions:** We created NLP-based tools and models and showed that they are clinically explainable and flexible across training data while maintaining consistent performance. We refined these tools by factoring in additional features and were better able to identify latent topics associated with readmission risk. In future works, this model will be used in a data analysis pipeline to train a readmission risk classifier.

**Keywords:** Readmission Risk, Electronic Health Record (EHR), Natural Language Processing (NLP), First-Episode Psychosis, Machine Learning Classification

**Disclosure:** Nothing to disclose.

### T209. Biomarker Study of Prodromal Schizophrenia Using Raman Spectroscopy

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*University of Pittsburgh, Pittsburgh, Pennsylvania, United States*

**Background:** Schizophrenia (SZ) is a common, potentially severe disorder for which few, if any reliable biomarkers are available. In particular, it is difficult to predict psychosis among individuals with prodromal features. Some serum-based assays to predict which individuals will convert to the full syndromal state are now available. Typically, panels of pre-selected proteins are used. Such analyses entail time consuming, reagent-based, labor intensive and relatively expensive analyses. We investigated the utility of Raman Molecular Imaging (RMI), an agnostic, reagentless technique that combines digital imaging with Raman Spectroscopy (RS). RS relies on the 'Raman effect', a characteristic shift in the wavelength of incident laser light scattered by individual molecules. Thus, solids and liquids produce characteristic spectral patterns with unique signatures that can be analyzed rapidly using advanced statistical techniques. While RS is used routinely in industry, microbiology and in biotechnological manufacturing processes, it is also being used increasingly in cell biological research, including cancer diagnosis and staging, analysis of atherosclerotic vascular disease, and in cellular differentiation. We investigated the feasibility of RS for biomarker studies of prodromal SZ.

**Methods:** First-degree relatives (offspring and siblings) of patients with SZ were evaluated and those who were diagnosed with schizophrenia over a 2-year follow-up were considered cases. They were compared with demographically matched individuals without personal/family history of psychosis. All participants completed the SCID, followed by consensus diagnoses (DSM IV criteria). Serum samples were obtained before SZ diagnosis from the cases (and from controls), stored at -80° C and thawed in batches, dried on aluminum coated slides overnight prior to data collection. RMI was performed using a Bruker Senterra Raman Microscope with 785 nm laser and using OPUS software at ChemImage Corp. in Pittsburgh, PA. Spectra were photobleached for 30 seconds before collecting a spectrum generated from three co-added 60 second integrations. Obvious outlier samples were removed, blind to group status. Each sample was represented by 12 spectra on average. Samples were run in randomized order and the operator was blinded to group status. We used partial least squares discriminant analysis (PLSDA), a supervised classification model, to seek group-wise discrimination. The performance of the PLS-DA model was evaluated by calculating sensitivity, specificity, and area under the receiver operating characteristic (ROC) curve.

**Results:** There were no significant demographic differences between the converters and control individuals (converters, n = 11, mean age 22.63 years, 54% women) and controls (n = 11, mean age 22.36 years, 45% women). All the included spectra from each sample were used in the analysis and discrimination characterized by ROC curve. The following estimates were obtained: Sensitivity- 72.7%, Specificity- 72.7%, Accuracy- 72.7%, Area under the curve (AUC): 0.645.

**Conclusions:** We demonstrate that RMI is a feasible approach for biomarker studies in SZ. Though it did not attain the conventional levels of significance in this small pilot study, the relatively high sensitivity and specificity estimates augur well for RMI analyses of larger samples, particularly comparisons between 'converters' and 'non-converters'. 'Head to head' comparisons with conventional serological assays and value added by combining RMI with other assay panels should be estimated. RMI is an agnostic, reagentless approach to serum-based prediction that shows promise as a diagnostic tool in SZ research.

**Keywords:** Raman Spectroscopy, First Episode Schizophrenia, Peripheral Biomarker

**Disclosure:** Nothing to disclose.

### T210. Deep Learning-Based Human Activity Recognition for Continuous Activity and Gesture Monitoring for Schizophrenia Patients With Negative Symptoms

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**Background:** Quantified evaluation and continuous monitoring of behaviour and symptoms associated with negative symptoms of schizophrenia has been a challenge in clinical trials. Wearable sensor technology provides new opportunities to tackle these

problems. In addition, the recent breakthrough in Deep Learning offers a novel approach for accurately inferring subjects' behaviour through wearable sensor signals. The combination of the two could make continuous remote monitoring of schizophrenia patients in a clinical trial setting a reality. However, to connect the sensor data with clinical observations and measures of motivated behaviour still requires development of advanced analytics and real-world validations.

**Methods:** We conducted a 3-way cross-over proof of mechanism (POM) study of a compound targeting negative symptoms of schizophrenia (Roche study BP29904); 33 patients with moderate negative symptoms were recruited (30 males; 21 Black / African American, 9 White, 3 Asian; mean age 36.6 ± 7 y; BNSS total score = 36.0 ± 3.6; PANSS NSFS = 22.8 ± 1.4; PANSS PSFS = 19.4 ± 1.8). Of these, 31 patients were provided with a GeneActiv© wrist-worn actigraphy device to record actigraphy data for 15 weeks. Negative symptoms were rated with the Brief Negative Symptom Scale (BNSS); Motivated behaviour was assessed with an effort choice task. To analyse the actigraphy data, we trained a 9-layer convolutional recurrent neural network using two public data sets containing wrist-worn acceleration data to infer the subjects' activities. The trained human activity recognition (HAR) model was tested with heldout validation data and achieved more than 95% of accuracy to separate the ambulatory (walking, stairs, cycling, jogging) activities from stationary activities (sitting, standing, lying down, hand work). We applied the HAR model on the collected actigraphy acceleration data of 31 schizophrenia patients from the BP29904 study and calculated active time ratio in patients' daily life. We also inferred the gesture events and gesture features based on the activities and acceleration signal using an empirically defined threshold on accelerometer signal from the wrist. To infer the gesture events, we extracted the time spans where the patients were predicted as stationary, while the standard deviation of the magnitude of the accelerometer signals exceeded an empirically-defined threshold of 0.1 m/s2. From derived gesture events we generated number of gesture events and the gesture power as gesture-related features. Correlations between active time ratio, gesture features, levels of negative symptoms and willingness to work for a high reward in the effort choice task during placebo treatment were calculated. Correlation measures were calculated using Spearman correlation and P values were corrected using Banjamini-Hochberg procedure.

**Results:** The patient adherence rate was high: average monitoring time per patient was 1,859 h, among which 25 patients have more than 1,000 h of actigraphy data collected. The active time ratio of the schizophrenia patients was significantly associated with the % high effort choice during the placebo period (PBO, r = 0.58, P = 0.002). The median daily gesture counts are also found to be negatively correlated with BNSS total score during PBO (r = -0.44, P = 0.03). Specifically, with for the diminished expression sub-score at the baseline during PBO, both median daily gesture counts and median gesture powers are negatively correlated with higher score (r = -0.42, P = 0.03 in both cases). We have also investigated the change of negative symptoms between placebo period versus the dosage period. We also observed negative correlation between the changes in the diminished expression sub-score and the changes in median gesture counts (r = -0.47, P = 0.07 at low dose vs PBO, r = -0.34, P = 0.21 at high dose vs PBO), as well as changes in median gesture power (r = -0.39, P = 0.12 at low dose vs PBO, r = -0.7, P = 0.01 at high dose vs PBO).

**Conclusions:** These analyses demonstrate the feasibility to the use of wrist-worn actigraphy for continuously monitoring clinically-relevant behaviour in a clinical trial setting. Associations with key dimensions of negative symptoms support their validity. The activity and gesture features derived from human activity recognition model shows promise for future clinical practice and drug development process. While we have seen encouraging correlations, a further validation study with more patients is still required to establish conclusive statistical evidence.

**Keywords:** Schizophrenia, Negative Symptoms, Biomarker, Actigraphy, Effort Choice Task

**Disclosure:** F. Hoffmann - La Roche, Employee, Stock / Equity

### T211. Methodological Issues and Publication Bias in Reporting Clinical Trials in Schizophrenia

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**Background:** Progress in developing drugs in medicine in general and in psychiatry in particular is plagued by in-appropriate changes in study design, changes in statistical methods, inaccurate reporting of data, and non-publication of studies. These might mislead the field and impede drug development. We examined 253 studies funded by the Stanley Medical Research Institute (SMRI) between the year 2000 and 2010 and looked at rates of publication, and differences between the protocols as proposed to the funding agency, and the published papers.

**Methods:** We reviewed all studies funded by SMRI from 2000 to 2010. We then used an online database (RedCap) to collect data on both protocols and related papers of the funded studies. We then compared outcome measures and enrollment numbers as proposed in the original protocols and then as published in the papers.

**Results:** Between the years 2000 and 2010, 253 studies were funded by SMRI. Of these studies, 12.3% were not completed. Of the studies completed, rates of publication ranged from 26%-73%, mean rates of publication was 46.3%. Time to publication after study completion ranged from 1 to 4 years (mean = 2.06 ± 0.83 years). We examined 96 studies that were completed and published and compared protocols to papers. Findings showed that 4.2% of the papers had a longer length of treatment compared to that proposed in the protocol, and 17.7% of the papers reported shorter duration of treatments than proposed. 37.5% of the papers had a sample size 10% or smaller than proposed. Of the papers analyzed, 28.1% presented a different primary outcome measure then proposed, and 16.7% reported a positive finding using a different primary outcome measure than proposed.

**Conclusions:** Changes of protocols during studies, imprecision of reporting, and non-publication of clinical trial data are common in SMRI funded studies, with rates similar to those reported in non-psychiatric fields. This is damaging for the field, potentially leading to misinterpretation of results leading to unnecessary exposure of patients to study procedures/placebo and to a waste of funds that might be used for more promising innovative compounds instead.

**Keywords:** Publication Bias, Schizophrenia, Methodologies, Clinical Trials

**Disclosure:** Nothing to disclose.

### T212. Inflammatory Markers are Associated With Psychomotor Speed in Patients With Schizophrenia Compared to Healthy Controls

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**Background:** Previous data have demonstrated that administration of inflammatory cytokines or their inducers leads to altered basal ganglia function associated with reduced psychomotor speed. Patients with schizophrenia (SCZ) exhibit psychomotor slowing and cognitive impairments in many domains. Increased inflammatory markers are seen in some patients with SCZ compared to healthy controls. Previous work has reported relationships between inflammatory markers and cognitive impairment, though most previous studies have evaluated a small number of inflammatory markers and/or a small number of cognitive tasks. We thus measured a broad array of inflammatory markers that have been shown to be related to SCZ in addition to a variety of psychomotor tasks. We hypothesized that there would be associations between inflammatory markers and psychomotor speed in SCZ subjects compared to healthy controls.

**Methods:** 43 patients with SCZ and 29 healthy controls (CON) were included in the study. All participants were recruited from the Atlanta Veterans Affairs Medical Center. The following inflammatory markers were measured: tumor necrosis factor (TNF), interleukin (IL)-1beta (IL-1b), IL-6, IL-10, monocyte chemoattractant protein 1 (MCP-1), IL-6 soluble receptor (IL-6sr), IL-1 receptor antagonist (IL-1ra), and TNF receptor II (TNFR2). Concentrations of inflammatory molecules were assessed in duplicate using high sensitivity multiplex bead-based assays (R&D Systems) and analyzed on a MAGPIX CCD imager (Luminex). Due to concerns for co-linearity between the inflammatory markers, principle components analysis (PCA) with varimax rotation was used to create factor loadings that were subsequently used in analyses. The following psychomotor tasks were used: Finger Tapping Task, Reaction Time Task, Symbol Coding, and Trail Making Test. T-tests and chi-square tests were performed to test for differences between diagnostic groups. Spearman's rank order correlations were used to test the associations between inflammatory markers and cognitive tasks. Finally, stepwise, backward linear regression models were used to determine the relationship between inflammatory markers and cognition.

**Results:** There were no differences in age, sex or smoking status between SCZ and CON, but the SCZ group had a higher proportion of black individuals (chi square = 4.511, $p = 0.034$). Performance was worse in SCZ than CON on the Finger Tapping Test ($p = 0.002$) as well as Symbol Coding and Trail Making Test (both $p < 0.01$). SCZ subjects had higher concentrations of IL-1RA compared to CON ($t = -2.004$, $p = 0.049$), but no other markers differed significantly between groups.

In SCZ subjects, higher levels of TNF ($p = 0.034$), IL-1b ($p = 0.047$) and IL-10 ($p = 0.027$) correlated with worse Finger Tapping performance. Similarly, higher levels of IL-1b ($p = 0.033$) and IL-10 ($p = 0.027$) correlated with worse Trail Making Test performance.

The PCA yielded three factors: a factor that included TNF, IL-10, MCP-1 (Factor 1), a factor that included IL-1b, IL-6, IL-1RA (Factor 2), and a factor that included IL6sr and TNFR2 (Factor 3). In linear regressions, the PCA factors predicted worse psychomotor and processing speed performance in models controlling for demographics, smoking, and Toxoplasma gondii IgG serointensity. Specifically, Factor 1 predicted worse performance on the Finger Tapping Task ($p = 0.029$) and Trail Making Test ($p = 0.012$). However, factor 3 predicted better performance on the Finger Tapping Task ($p = 0.01$), Reaction Time Task ($p = 0.000$), and Symbol Coding ($p = 0.034$).

Fewer and less consistent associations were found between inflammatory markers and cognition in CON subjects in both correlations and linear regressions.

**Conclusions:** Psychomotor speed and processing speed were significantly worse in SCZ than CON subjects. Inflammatory markers were more predictive of psychomotor slowing in SCZ subjects than in CON. This finding is consistent with prior studies demonstrating that inflammatory stimuli alter basal ganglia function associated with reduced psychomotor speed as well as a recent study demonstrating similar findings in patients with major depressive disorder. As such, this study provides further support that peripheral inflammatory markers in patients with SCZ may contribute to psychomotor slowing. Psychomotor speed may serve as a relevant outcome variable for future studies targeting inflammatory mediators to treat patients with neuropsychiatric disorders such as SCZ.

**Keywords:** Schizoprenia, Cytokines, Psychomotor Speed, Inflammation, Cognition

**Disclosure:** Nothing to disclose.

## T213. Efficacy and Safety of an Asenapine Transdermal Patch (Asenapine Transdermal System, HP-3070) in the Treatment of Adults With Schizophrenia: A Phase 3 Randomized, Double-Blind, Placebo-Controlled, 6-Week, Inpatient Study

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**Background:** Asenapine is a second-generation antipsychotic currently marketed in the US in sublingual form for the treatment of schizophrenia and bipolar mania/mixed episodes. HP-3070, asenapine transdermal system, is a once-daily patch for the treatment of schizophrenia in adults. It was designed with certain advantages over sublingual asenapine, including reduced risk of oral hypoesthesia and dysgeusia, once-daily dosing, lack of food or drink restrictions, and steadier absorption and plasma levels of asenapine. Low-dose and high-dose HP-3070 were designed to be equivalent in terms of overall medication exposures to sublingual asenapine 5 mg BID and 10 mg BID, respectively. Once approved, HP-3070 will be the first transdermal antipsychotic and will provide patients and caregivers a novel treatment formulation.

**Methods:** This international Phase 3, randomized, double-blind, fixed-dose, placebo-controlled, in-patient study was conducted in 59 centers. A Screening/Run-in Period of 3-14 days was followed by a 6-week Double-Blind Treatment Period and 30-day Follow-up. The study enrolled persons with an acute exacerbation of schizophrenia with screening and baseline Positive and Negative Syndrome Scale (PANSS) total score ≥ 80 with scores ≥ 4 on at least 2 out of 4 predefined PANSS positive subscale items, and a Clinical Global Impression–Severity of Illness Scale (CGI-S) score ≥ 4. Patients were excluded if they were known non-responders to asenapine or if they were resistant/refractory to antipsychotic treatment. Eligible, enrolled patients were randomized 1:1:1 (HP-3070 low dose, HP-3070 high dose, and placebo).

The primary objective was to evaluate efficacy of HP-3070 vs placebo by change from baseline (CFB) in PANSS total score to Week 6. The key secondary objective was CFB in CGI-S score at Week 6 vs placebo. Safety assessments included treatment-emergent adverse events (TEAEs), CFB in clinical laboratory results and vital signs, assessment of dermal safety, and results of the Barnes Akathisia Rating Scale (BARS), Abnormal Involuntary Movement Scale (AIMS), and Simpson-Angus Scale (SAS).

The primary efficacy variable was analyzed using mixed model repeated measures (MMRM) with CFB in PANSS total score as the repeated dependent variable, and country, treatment group, visit, treatment by visit interaction, and the baseline PANSS total score as covariates. The p values were adjusted for multiple comparisons. If either or both active doses were positive in the primary efficacy analysis, analysis for the key secondary endpoint was performed using Hochberg-based gatekeeping procedure to limit overall Type I error rate to < 0.05 by taking into account multiple doses and multiple primary and key secondary endpoints.

**Results:** A total of 616 patients were randomized and 486 patients completed the study. The discontinuation rate was 23.3%, 18.6%, and 21.4% for HP-3070 high dose, HP-3070 low dose, and placebo groups, respectively. For each group, withdrawal of consent and AEs were the most common reasons for discontinuation. Demographics and baseline characteristics were generally well balanced among the treatment groups. The majority of subjects were male (60.6%), white (76.0%), and aged < 55 years (83.0%).

For the primary endpoint and key secondary endpoint, both doses of HP 3070 (both doses) were statistically significantly better than placebo (Hochberg adjusted $p < 0.05$). For PANSS total score, the least squares mean (LSM) estimates with standard error (SE) of the treatment comparison (HP-3070 vs placebo) for the CFB at Week 6 was -4.8 (1.634; 95% CI: -8.06, -1.64; $p = 0.003$) and -6.6 (1.630; 95% CI: 9.81, 3.40; $p < 0.001$) for the high and low dose groups, respectively, compared with placebo. For CFB CGI-S score at Week 6, the LSM (SE) for the treatment comparison (HP 3070 vs placebo) were 0.4 (0.100; 95% CI: 0.55, 0.16; $p < 0.001$) for the high dose group and 0.4 (0.099; 95% CI: 0.64, 0.25; $p < 0.001$) for the low dose group.

There were no deaths and no serious AEs related to study treatment. Most TEAEs were mild or moderate in severity. The most frequently reported group of TEAEs in patients who received HP-3070 were nervous system disorders (24.0% and 21.6%, for high and low dose groups respectively vs 12.6% for placebo) with headache and extrapyramidal disorder being the most frequent. Placebo patients had higher rates of psychiatric disorders (incidence of 15.7% and 17.6% for HP-3070 high and low dose groups respectively, vs 24.3% for placebo) with insomnia and anxiety being most common. TEAEs at the patch application site were higher in the HP-3070 groups (14.2% and 15.2% in the high and low dose groups, respectively) compared with the placebo group (4.4%); most of these events were mild or moderate in severity. TEAEs leading to discontinuation of study treatment were reported for 7.8%, 4.9%, and 6.8% of subjects in the HP-3070 high dose, HP-3070 low dose, and placebo groups, respectively. Discontinuations due to application site reactions or skin disorders (urticaria, pruritus) was low ( ≤ 0.5% per treatment group). There were no marked mean CFB results for vital signs or ECG parameters, nor treatment differences observed for the EPS assessments (SAS, AIMS, BARS).

**Conclusions:** HP-3070 is efficacious for the treatment of schizophrenia in adults and met both its primary and key secondary endpoints for both the low and high doses. HP-3070 was safe and well tolerated, showing a similar systemic safety profile to sublingual asenapine.

**Keywords:** Schizophrenia, Antipsychotics, Schizophrenia Novel Treatment, CNS Clinical Trials

**Disclosure:** Noven Pharmaceuticals, Inc., Grant, Otsuka, Grant, Janssen, Grant, Sunovion, Grant, Takeda, Grant, Allergan, Grant, Acadia, Grant, Alkermes, Grant, Blackthorn, Grant, Boehringer Ingelheim, Grant, Lundbeck, Grant, Roche, Grant, IntraCellular, Grant

### T214. Rapid Augmentation of Antipsychotic Drugs by Sodium Nitroprusside (SNP): Behavioral Assessment and Effect on Brain Dopaminergic Transmission in Rats

Abstract not included.

### T215. Prenatal THC Exposure Permanently Disturbs Kynurenic Acid and Glutamate Levels and Amplifies the Responsivity to an Acute Kynurenine Challenge in the Rat Prefrontal Cortex

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**Background:** Throughout the world, cannabis remains one of the most widely used illicit drugs during pregnancy (Porath-Waller, 2015). The main psychoactive component of marijuana (delta9-tetrahydrocannabinol, THC) passes through the placenta, and its use is correlated with early physiological effects in the offspring. Neurobehavioral and cognitive impairments have been reported in several longitudinal studies on children and adolescents prenatally exposed to marijuana (Calvigioni et al., 2014), and a link to psychiatric disorders has been proposed (Jutras-Aswad et al., 2009; Mathews et al., 2014). Prenatal exposure to cannabinoids induces cognitive deficits in rat offspring (Ferraro et al., 2009) and is associated with alterations in cortical/hippocampal glutamate and GABA levels (Antonelli et al., 2005, Beggiato et al., 2017). Interestingly, the deleterious effects of cannabinoids on cognitive functions are similar to those observed in adult rats prenatally exposed to (L)-kynurenine (KYN), which is the direct bioprecursor of kynurenic acid (KYNA), a neuroactive metabolite of tryptophan degradation (Pocivavsek et al., 2014).

**Methods:** We investigated whether alterations in KYNA levels in the rat brain might play a role in the long-term consequences of prenatal cannabinoid exposure. Pregnant Wistar rats were treated daily with THC [5 mg/kg or vehicle (sesame oil) by oral gavage] from gestational day (GD) 5 through GD 20. One adolescent [postnatal day 35-45] and one adult male rat per litter was then used to determine the extracellular levels of KYNA and glutamate before and after a challenge with KYN (5 mg/kg i.p.) by in vivo microdialysis in the medial prefrontal cortex (mPFC).

**Results:** Compared to vehicle-treated controls, extracellular basal KYNA levels were higher in adolescent and adult rats that had been prenatally treated with THC ($p < 0.01$; $p < 0.05$, respectively). These rats also had lower extracellular glutamate levels than respective controls ($p < 0.01$; $p < 0.05$, respectively). Following a challenge with KYN, extracellular KYNA levels increased in both adolescent groups (i.e. vehicle- and THC-treated; $p < 0.05$). Interestingly, this effect was more pronounced in adult rats which had been prenatally exposed to THC. KYN also caused a trend towards a reduction in extracellular glutamate levels in vehicle-treated adolescent and adult rats.

**Conclusions:** We propose that these permanent alterations in KYNA and glutamate signalling in the mPFC of prenatally THC-exposed rats could be relevant for cognitive dysfunction. Our results are also in line with the hypothesis that a "double-hit" may precipitate psychiatric disorders such as schizophrenia later in life.

**Keywords:** Glutamate, Kynurenic Acid, Kynurenine, Prenatal, THC

**Disclosure:** Nothing to disclose.

### T216. Dexmedetomidine (Dex) and Agitation Part 1: Sublingual Administration Improves Rat Agitation/Aggression Behaviors

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**Background:** Agitation is central to various neuropsychiatric disorders such as Schizophrenia, Dementia, and Bipolar disorders and represent a significant challenge to physicians and caregivers. The potential for escalation to aggressive behaviour, putting patients and physicians at risk, makes it imperative to address agitated behaviour rapidly and efficiently and in a non-invasive manner if possible. In standard practice, antipsychotic agents like haloperidol are used to produce a rapid sedative effect, but the route of administration (IM/IV) of these drugs poses challenges in

agitated patients along with their associated side effects profile. On the other hand, the non-invasively administered 'Adusave' (inhaled loxapine), is associated with a high incidence of side effects and carries a black box warning for bronchospasm and increased mortality in elderly patients. Hence, a high unmet need exists for a rapidly acting, safe interventions that could be administered non-invasively to calm and stabilize mild to moderately agitated patients. Additionally, it is also important to effectively treat the underlying neurochemical disturbances associated with agitation. Elevated norepinephrine (NE) signalling and increased NE concentrations in CSF in various neuropsychiatric diseases strongly suggest that NE regulation may ameliorate the associated clinical condition of agitation and aggression. Therefore, Dex, (a selective alpha 2 adrenergic agonist) was investigated via a non-invasive route (sublingual) of administration to effectively treat agitation, which can decrease central NE release and noradrenergic tone on various behavioural parameters of agitation and aggression in rats. A PK/PD correlation following drug administration was also derived following experimentation.

**Methods:** For the behavioural study, the rat resident-intruder model, an established model for agitation and aggression was used. The resident rats were treated with different doses of Dex (0.3, 0.5, 1 & 1.5 μg/kg, doses which do not cause changes in rat heart rate and blood pressure) administered sublingually, 15 min prior to introduction of intruder rat in their home cage. The behaviour of each resident rat was video recorded and quantified using varying parameters of agitation & aggression such as biting, chasing, sniffing and attacking. In addition, neutral behaviours such as grooming, and exploration were also evaluated. For PK-PD correlation, plasma concentrations of dexmedetomidine were determined at varying time points after sublingual administration of the drug and correlated to the PD effect.

**Results:** Sublingually administered Dex significantly reduced the agitation and aggression in resident rats at doses of 1, 1.5 & 3 μg/kg compared to vehicle control. Doses from 0.5-1.5 ug/kg did not alter the neutral behaviours in animals. The plasma drug concentrations also correlated well with the pharmacodynamic effect of the drug.

**Conclusions:** Sublingually administered Dex can effectively treat rat agitation/aggression without affecting neutral behaviours or producing overt sedation. This is the first study to observe the effect of sublingually administered (non-invasive) Dex in improving agitation and aggressive behaviour in animals. Further, clinical studies are underway to evaluate the effect of this drug in a sublingual film (BXCL501) in producing calming behaviour in agitated neuropsychiatric patients.

**Keywords:** Agitation, Aggression, Alpha2 Adrenergic Receptors

**Disclosure:** Frank Yocca, Employee, Sameer Sharma, Employee, Manisha Chugh, Employee, Krishnan Nandabalan, Employee

## T217. Ca2 + Channel Abnormality Following NMDA Receptor Deletion in Cortical GABA Neurons

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**Background:** Cortical NMDAR hypofunction has been used to model the symptoms of schizophrenia. The parvalbumin (PV) expressing fast-spiking (FS) GABAergic interneurons are a prime target in this model. These cells provide perisomatic inhibition and mediate synchronous oscillatory activity. Abnormal neuronal synchrony is observed in schizophrenia; however, the underlying cellular mechanisms are not fully understood. Our mutant mice with postnatal deletion of the NMDAR subunit GluN1 predominantly in forebrain interneurons, a majority of which is PV containing, displayed impairments in in vivo synchronous membrane potentials by EEG (Nakao & Nakazawa, 2014). We sought to identify the critical cellular mediators involved in abnormal oscillations oscillations leading to cognitive dysfunction and focused our attention on the Cav2.1 channels (P/Q type), since they are highly expressed in PV-positive neurons and are responsible for FS-mediated fast GABA release onto excitatory neurons. To test whether such impairments may be due to dysfunction of P/Q type Ca2 + channels, we performed experiments in acute slices obtained from the prefrontal cortex of mice.

**Methods:** Using dual whole-cell patch clamp recordings, we tested synchronous inhibitory postsynaptic currents (IPSC). Medial PFC (mPFC) slices were prepared from Ppp1r2-cre( + /-)/GluN1 (f/f) mutant mice, Ppp1r2-cre( + /-)/Cacna1a (f/f) mutant mice, or their floxed control littermates, male and female, at 4-6 weeks old. Spontaneous IPSCs were recorded from pairs of layer II/III pyramidal neurons in the mPFC. To activate the Cav2.1/2.2 channel pharmacologically, its agonist GV-58 (50-100 μM) in combination with K + channel blocker 3,4-diaminopyrimidine (DAP, 3 μM) was bath-applied to the recording chamber. Using a custom written analysis program in R, the synchrony was measured as the percentage of IPSC events that had overlapping peak IPSC amplitudes within a 7-ms window.

**Results:** In 14 pairs of cells tested from control mice (from 6 female and 4 male mice), we determined that on average, 27.2 ± 0.94% of the IPSC events in the entirety of the recording were synchronous between the pairs. However, in the mutant mice (from 6 female and 7 male), the synchrony was lowered to 19% as measured in 14 pairs of cells ($p < 0.001$, t-test). Further evidence for impaired GABAergic transmission in the mutant mice was seen in the lowered frequency (Ctrl: 11.9 vs Mut: 9.1 Hz, $p < 0.01$ t-test) and reduced amplitude (Ctrl: 30.4 vs Mut: 25.1 pA, $p < 0.05$ t-test) of the IPSC events. Together, this data showed that the number and the size of GABA released from presynaptic terminals was affected by GluN1 KO. Further, this also impaired the ability of interneurons to synchronize their inhibition of pyramidal cells.

Next, we used the Cav2.1/2.2 channel agonist GV-58 (50-100 μM) in combination with K + channel blocker 3,4-diaminopyrimidine (DAP, 3 μM for reliable increase of AP duration). Co-administration of GV-58 and DAP is required for enhancing depolarization of the cell because GV-58 preferentially affects Ca2 + channels in open conformation. GV-58's effect was tested in a subset of cell pairs where IPSC frequency was measured (Ctl: $n = 5$ pairs from 3 female and 2 male mice; Mut: $n = 7$ pairs from 2 female and 5 male mice) GV-58 application significantly enhanced GABAergic transmission in both the control and the mutant group, as seen in the elevated IPSC frequency measure (Ctl: 10.6 vs GV: 13.85 Hz, $p < 0.01$ paired t; Mut: 9.6 vs GV-58: 12.6 Hz, $p < 0.01$ paired t-test). GV-58 also increased the percentage of IPSC synchrony in the mutant mice significantly (baseline: 19.8% vs GV-58: 25.6%, $p < 0.01$ paired t-test). In the control group however, the synchrony was not different before and after application of GV-58 (baseline: 26.7% vs GV-58: 29.4%, $p = 0.23$ paired t-test). This suggests that 1. IPSC synchrony may be independent of the frequency of the IPSC events; 2. In slices, the ceiling for IPSC synchrony may be 27-30%; 3. GV-58 through its action on P/Q channels may rescue the impairment in inhibition in GluN1 mutant mice by increasing GABAergic transmission and thereby network synchronization primarily through PV neurons. Separately, in a PV-Cre mediated GluN1 KO mouse that we tested where onset of knockout may be delayed, synchrony was not impaired [29.5 ± 2.6 %; $n = 5$ pairs, from 1 male and 3 female mice].

Finally, we analyzed a novel conditional knockout mouse strain of Cacna1a gene, encoding Cav2.1 (P/Q-type) channel pore subunit. In these animals, P/Q channel is knocked out in about 50% of GABAergic interneurons in a Cre dependent manner (using

the Ppp1r2cre line). In 4 pairs of cells tested, the frequency (avg 10.4 Hz) and amplitudes (avg 25.6 pA) of sIPSC events were not significantly different from the control group. However, the synchrony of the IPSC events between pairs of pyramidal cells was similar to the GluN1 mutant mice at 19.6%. Co-application of GV-58 and DAP increased the synchrony to 28.8 % ($p = 0.05$, paired t-test). Together, these data suggest that loss of P/Q channel impairs synchronous GABAergic events in mPFC and elevating their function may provide a mechanism for rescuing gamma oscillation deficits in the GluN1 mutant mice.

**Conclusions:** We showed that synchronous events of sIPSCs from pairs of layer II/III pyramidal neurons are diminished when either NMDAR subunit Grin1 or Cav2.1 channel Cacna1a is genetically ablated in ~50% of cortical GABA neurons. This change may also depend on the time of onset of knockout. The rescue effect of GV-58 Cav2.1 channel agonist on these measures in both mutants, may suggest down-regulation of Cav2.1 channels in the NMDAR-deleted PV-positive FS neurons.

**Keywords:** Gamma Oscillation, Voltage-Gated Calcium Channel, NMDA Receptor

**Disclosure:** Nothing to disclose.

### T218. Prenatal Kynurenine Elevation in Rats: Sex Differences in Hippocampal-Prefrontal Mediated Learning Impairments

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**Background:** Distinct abnormalities in kynurenine pathway metabolism have been reported in various psychiatric disorders, including schizophrenia (SZ). Kynurenic acid (KYNA) is an endogenous antagonist of α7 nicotinic acetylcholine (α7nACh) and NMDA receptors and increases in brain KYNA have been implicated in the pathology of SZ. Based on the neurodevelopmental hypothesis of SZ etiology, we have developed a model to study the KYNA hypothesis of SZ (Pocivavsek et al., Psychopharmacology, 2014). The bioprecursor to KYNA, kynurenine (kyn), is fed to pregnant rat dams the last week of embryonic gestation (control: ECon; kyn-treated: EKyn). Assessing only male offspring, we have previously determined that tissue KYNA levels remain increased in the hippocampus and prefrontal cortex of EKyn animals and EKyn offspring display learning and memory impairments, similar to cognitive dysfunctions that are core to SZ psychopathology. As sex differences in the prevalence and severity of the illness, including impact on cognition, have been observed, we presently investigated sex differences in hippocampal and prefrontal cortex-mediated learning in adult EKyn offspring.

**Methods:** For the prenatal paradigm, 100 mg of L-kynurenine sulfate was added daily to chow fed to pregnant Wistar rats from embryonic day (ED) 15 to ED 22. Brain tissues were collected from male and female offspring in adulthood (postnatal day 56-85). KYNA was measured in the hippocampal and prefrontal cortex samples ($N = 6 – 12$ litters per sex per group). Separate cohorts of offspring were tested in the Barnes maze ($N = 16 – 20$ per sex per group). After six learning trials (two trials per day, three testing days), the location of the Barnes maze escape box was moved to engage prefrontal-hippocampal circuitry during the reversal trial.

**Results:** KYNA in the hippocampus and prefrontal cortex was significantly elevated in adult male EKyn offspring compared to controls ($P < 0.05$) but remained unchanged in the EKyn females. Male EKyn offspring displayed significant impairments, evidenced as increased latency to find the escape box and increased errors, during the second acquisition day of the Barnes maze ($P < 0.05$). Of note, female EKyn offspring were not similarly impaired during acquisition. In female offspring, there was a minor delay in task acquisition, assessed as slightly increased escape latency across days, compared to ECon females and no significant differences in number of errors within acquisition days. After training, the location of the Barnes maze escape box was moved to engage prefrontal-hippocampal circuitry during the reversal trial. Both sexes of EKyn offspring were significantly impaired, taking longer to find the new escape box ($P < 0.05$), making more errors ($P < 0.05$), and also entering the previous escape box location more frequently than ECon offspring ($P < 0.001$).

**Conclusions:** Taken together, our data demonstrate that continuous elevation of KYNA levels during the last week of gestation is sufficient to cause long-lasting effects reminiscent of SZ in adult male offspring (increased brain KYNA, impaired hippocampal and prefrontal learning) and more subtle impairments in adult female offspring (no significant increase in brain KYNA, minor spatial learning deficits, impaired prefrontal-mediated reversal learning). In conclusion, the identified sex differences further support our prenatal kynurenine treatment in rats as an attractive tool to study the role of the kynurenine pathway in the pathophysiology of schizophrenia.

**Keywords:** Schizophrenia-like Behavior, Prenatal Exposure, Kynurenine Pathway, Kynurenic Acid, Sex Difference

**Disclosure:** Nothing to disclose.

### T219. Chronic Cell-Type Specific Modulation of Nucleus Accumbens Medium Spiny Neuron Activity Precipitates Stress-Like Effects on Sleep Architecture

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**Background:** Changes in sleep (insomnia, hypersomnia) are well known to accompany stress-related psychiatric conditions including Major Depressive Disorder (MDD) and Post-Traumatic Stress Disorder (PTSD), both of which are defined by depressive-like features. Research from our lab and others has demonstrated that the NAc is important for the development of stress-related psychiatric conditions. Specifically, changes in CREB, ΔFosB and other genes within the NAc dramatically affect susceptibility to chronic stress related behavioral phenotypes. Chronic stress leads to elevations in CREB and overexpression of CREB leads to anxiogenic and depressive-like behaviors, whereas suppression of CREB function produces anxiolytic-like effects. Within the NAc, enhanced CREB activity leads to increases in the expression of dynorphin, an endogenous kappa opioid receptor (KOR) ligand that is expressed in dopamine D1 receptor- expressing medium spiny neurons (MSNs). Molecular or activity-related changes in D1- and D2-expressing neurons have been shown to modulate core features of depressive illness, such as anhedonia (reduced ability to experience pleasure) or social avoidance following stress. Specifically, changes in the molecular and firing properties of D1 MSNs have been observed following to chronic social defeat stress (CSDS) and correlated with susceptibility to developing these depressive behaviors. Our lab recently demonstrated that CSDS not only produces many depression-like behaviors, but also causes increases in paradoxical sleep (called REM sleep in humans). This effect is blocked by the administration of JDTic, a KOR antagonist, further implicating D1 MSN's in stress-precipitated alterations in paradoxical sleep.

**Methods:** To modulate the activity of D1 and D2 MSNs, we used mouse lines expressing Cre-recombinase in D1 MSNs (GENSAT FK-150) or D2 MSN's (GENSAT ER44). Heterozygous littermates were

bilaterally infected with viral vectors (AAV5-DIO-) encoding cre-dependent excitatory (hM3Dq) or inhibitory (hM4Di) DREADDs (designer receptors exclusively activated by designer drugs) tagged with an mCherry reporter; control animals were infected with virus encoding only mCherry. All mice were implanted with wireless EEG telemetry devices (DSI) that allow continuous monitoring of EEG/EMG over weeks to months. DREADDs were activated by chronic administration of clozapine in drinking water (0.1 mg/kg/8 h). Mice were monitored for a 5-day baseline period, then received 10 days of chronic DREADD activation, followed by a 5-day washout period. Data were analyzed using ANOVAs and post hoc tests.

**Results:** Chronic inhibition of D1 MSNs through Gi DREADD lead to a selective increase in the time spent in paradoxical sleep ($P < 0.01$) without affecting time spent in slow wave sleep or wake. Chronic activation of D1 MSNs through Gq DREADD lead to moderate decreases in paradoxical sleep ($P < 0.05$) without affecting time in slow wave sleep or wake. Theses effects were maintained for at leastfive days, as they remained evident during the washout period (P's < 0.05-0.01) suggesting that activation or inhibition of D1 MSNs leads to persistent effects on sleep architecture.

**Conclusions:** Changes in sleep architecture are an important component of stress-related disorders. Specific changes to D1 MSNs have been implicated in susceptibility to stress-precipitated dysfunction, and here we suggest they may be crucial for regulation of stress-precipitated changes in sleep. Chronic inhibition of D1 MSNs leads to increases in paradoxical sleep while chronic activation leads to decreases in paradoxical sleep. Importantly, this recapitulates effects of CSDS on paradoxical sleep without changing slow wave sleep or wakefulness. Importantly, changes in REM sleep are frequently observed in humans with depression and PTSD. Our data provide the basis for a circuit-based model whereby chronic stress may change D1 MSN activity leading to alterations in sleep architecture similar to those seen in humans with stress-related illnesses.

**Keywords:** Chronic Social Defeat, Sleep, Nucleus Accumbens, Depression

**Disclosure:** Nothing to disclose.

## T220. Prevalence and Impact of Psychiatric Symptoms in an Undiagnosed Diseases Program

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**Background:** A decade ago, the NIH launched an undiagnosed diseases program to investigate mysterious, often multi-system diseases. At this time, there are no published reports describing the prevalence and impact of the presence of psychiatric symptoms or diagnoses in patients with undiagnosed diseases. To evaluate the presence of psychiatric symptoms or diagnoses in a cohort of clinical patients seeking care at the Emory Special Diagnostic Service (ESDS). We hypothesized there would be a high prevalence of psychiatric symptoms in this population and that patients who endorse psychiatric symptoms would have decreased quality of life and greater functional impairment.

**Methods:** This is a cross-sectional, retrospective analysis of 247 patients from the Emory Special Diagnostic Clinic seen between February 7, 2014 and May 31, 2017. The sources for data included the Emory Health History Questionnaire that had the Work and Social Adjustment Scale (WSAS) and Quality of Life Enjoyment and Satisfaction Questionnaire – short form (Q-LES-Q) embedded in it, medical records from referring physicians, and the comprehensive, standardized ESDS template. The primary outcomes were a broad definition of a psychiatric symptoms derived from Emory Health History Questionnaire that included questions about mental health history and current psychiatric symptoms or diagnoses, report of psychiatric symptoms or diagnoses in the medical record, or a narrow definition of preexisting psychiatric disorder based on a psychiatric diagnosis confirmed in the medical record. The aforementioned WSAS and Q-LES-Q short form were also assessed.

**Results:** One hundred and seventy-eight out of 247 patients (72.5%) evaluated at the ESDS met the broad criteria for having a psychiatric diagnosis while 60 out 247 patients (24.6%) met the narrow criteria for having a psychiatric diagnosis. Patients meeting criteria for a broad definition of a psychiatric diagnosis had a significantly diminished Q-LES-Q scores ($45.27 + /- 18.63$) versus patients with no psychiatric symptoms ($62.01 + /- 21.57$, $t = 5.60$ $df = 225$ $p < 0.0001$). Patients meeting criteria for the narrow definition of psychiatric diagnoses also had significantly diminished quality of life ($45.16 + /1 17.28$) as versus those who did not meet criteria for psychiatric diagnoses ($51.85 + /- 21.54$, $t = 2.11$ $df = 225$, $p = 0.036$). Patients who met the broad definition of psychiatric symptoms were significantly more impaired than patients without any psychiatric diagnoses on all 5 items of the WSAS, while patients who met the narrow definition of a psychiatric diagnoses did not differ from those without a psychiatric diagnosis on any WSAS item.

**Conclusions:** The presence of psychiatric symptoms is high in this population of patients evaluated in the clinic for undiagnosed disorders. Employing the broadest definition of psychiatric symptoms, patients had significant decreases in both quality of life, work and functioning as compared to those individuals with no psychiatric symptoms. This suggests that the assessment of psychiatric symptoms as part of the evaluation of individuals with undiagnosed disorders may have important diagnostic implications.

**Keywords:** Psychosomatic Medicine, Comorbidity, Mind & Body Approaches

**Disclosure:** Nothing to disclose.

## T221. Symptom Improvement Following Cognitive Behavioral Therapy in Irritable Bowel Syndrome Results in Changes in Brain Gut Microbiome Axis

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**Background:** While there is growing preclinical evidence for a role of bidirectional communication between the gut microbiome and the brain in the regulation of emotional and social behaviors, evidence from studies in human brain disorders remains limited, and causality has not been established. Irritable bowel syndrome (IBS) is a disorder of brain gut interactions and an altered gut microbiome (dysbiosis) has been implicated in symptom generation. Structural and functional alterations in the salience and sensorimotor networks in have been reported in IBS. Cognitive behavioral therapy (CBT) is one of the most effective therapies for IBS, suggesting a prominent role of the brain in IBS pathophysiology. We recently showed in a large randomized controlled trial that CBT significantly reduces IBS symptom severity scores (IBS-SSS), and that this improvement was maintained 6 months after therapy completion (Lackner et al, 2018). By using an effective

brain-targeted therapy, we tested the hypotheses that 1) CBT-related symptom improvement is related to both brain and microbiome features and 2) the brain plays an important role in the observed dysbiosis in IBS.

Aims: To determine whether CBT associated symptom improvement in IBS is associated with changes in resting-state functional connectivity of the brain, and if these brain changes result in a normalization of gut microbiome composition and function.

**Methods:** Brain imaging: Functional resting state magnetic resonance imaging (rs-fMRI) was measured in 120 subjects (97 Females) at baseline and two weeks post treatment from 80 IBS patients, who were randomized to a clinic-based CBT (n = 47) or home-based CBT (n = 33). fMRI data were analyzed using the CONN toolbox. The brain was parcellated into 165 total regions of interest using the Destrieux and Harvard-Oxford atlases, and a 165 × 165 adjacency matrix was obtained for each subject containing Fisher transformed correlation values (Z) between each ROI. Two GLMs controlling for age and sex were used to conduct a paired t-test on ROI-to-ROI results to determine differences in rs-FC before and after CBT in responders (N = 51) and non-responders (N = 29). Significance was set after correcting using the false-discovery rate (FDR) at $q < 0.05$. Gut microbiome: In a subset of IBS patients, stool samples were collected at baseline and after 12 weeks of treatment from 49 IBS patients (80% female; 37% IBS-C, 47% IBS-D) randomized to clinic-based CBT (n = 19) or home-based CBT (n = 15).16 S rRNA sequencing and metabolomics analysis (HD4, Metabolon) were performed. Differential abundance was determined using multivariate negative binomial or general linear models adjusting for IBS subtype and sex.

**Results:** 1. Clinical Changes: Two weeks after 10-week acute treatment phase, CBT-treated patients showed a large improvement on the IBS-SSS ($t(77) = -9.44$, $p < .0001$), with 51(42.5%) subjects ("Responders") showing a greater than 50-point decrease on the IBS-SSS. 2. Brain Connectivity: Responders to CBT showed a decrease in rs-FC between regions of the salience, somatosensory, and emotion regulation networks. Specifically, the brainstem and bilateral temporal cortices (all $q = 0.04$), anterior insula (aINS) and medial prefrontal cortex (mPFC; $q = 0.01$), amygdala to lateral temporal cortex ($q = 0.04$), posterior insula and superior temporal gyrus ($q = .02$), and between the aINS and anterior mid-cingulate cortex ($q = 0.04$). On the other hand, non-responders to CBT showed a decrease in rs-FC between the dorsal posterior cingulate cortex and the ventral posterior cingulate cortex ($q = 0.04$). 3. Microbiome: Baseline microbiota composition was significantly associated with IBS-SSS response ( > 50 decrease) in CBT-treated patients at two weeks and 3 months post-treatment ($p = 0.016$, 0.029). CBT responders had increased Clostridiales (including the genera: Roseburia, Lachnobacterium, and unclassified Lachnospiraceae ($q = 0.03$, 0.04, 0.04) and decreased Bacteroidales (including genera Bacteroides, Parabacteroides, and Prevotella ($q = 0.01$, 0.0002, 0.04), and increased stool serotonin levels. CBT shifted relative abundances towards higher Bacteroides only in responders. A classifier generated from 11 genera had a high predictive accuracy CBT response.

**Conclusions:** The association of pre-treatment fecal microbiota (high Clostridiales to Bacteroidetes abundance ratio) with CBT response, together with a CBT-induced shift from Clostridiales to Bacteroidetes) in responders is consistent with an important role of the brain in modulating the gut microbiota. Brain findings suggests that increased connectivity of networks that contribute to IBS symptoms (Mayer et al. 2015) progress towards normalization after CBT, and this normalization is associated with a shift in the relative abundances of major microbial genera. The correlation of increased fecal serotonin and Clostridiales in responders is consistent with prior literature linking Clostridiales to luminal serotonin release (Jano et al, 2016). These results demonstrate significant biological changes within the brain gut microbiome axis related to CBT-induced symptomatic improvement. Fecal microbial analyses may be useful in identifying patients who most benefit from CBT.

**Keywords:** Brain, Gut Microbiome, Functional MRI (fMRI), Cognitive Behavioral Therapy, Gut-Brain Axis

**Disclosure:** Nothing to disclose.

## T222. Use of the Mouse Head Twitch Response to Investigate the Behavioral Effects of Phenylalkylamine Hallucinogens and Their Tetrahydrobenzodifuran ("FLY") and Benzodifuran ("DragonFLY") Analogs

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**Background:** Hallucinogen use by humans has remained relatively stable over the past decades but has increased in recent years. One factor contributing to increasing use of hallucinogens is a marked increase in the availability and number of "designer" hallucinogens that are sold over the internet. There are now hundreds of different hallucinogens available, with new substances appearing regularly, some being associated with significant toxicity and fatalities. Examples include 2-(8-bromo-2,3,6,7-tetrahydrobenzo[1,2-b:4,5-b′]difuran-4-yl)ethan-1-amine (2C-B-FLY) and 1-(8-bromobenzo[1,2-b;4,5-b′]difuran-4-yl)-2-aminopropane (Bromo-DragonFLY, DOB-DFLY), which are structurally rigid analogs of phenylalkylamine hallucinogens. Although some rigid compounds such as DOB-DFLY reportedly have higher potency than their non-rigid counterparts, it is not clear whether the same is true for 2C-B-FLY and other tetrahydrobenzofurans. In the present study, the head twitch response (HTR), a 5-HT2A receptor-mediated behavior induced by serotonergic hallucinogens, was used to assess the effects of 2,5-dimethoxy-4-bromoamphetamine (DOB) and its α-desmethyl homologue 2,5-dimethoxy-4-bromophenethylamine (2C-B), as well as their benzodifuranyl and tetrahydrobenzodifuranyl analogs, in C57BL/6 J mice. In addition, to assess the relationship between hallucinogen potencies in mice and humans, a linear regression analysis was used to compare HTR-derived ED50 values with human potency data reported in the literature.

**Methods:** The HTR was assessed using a head-mounted magnet and a magnetometer detection coil. Briefly, male C57BL/6 J mice (6-8 weeks old) were anesthetized, a small incision was made in the scalp, and a small neodymium magnet was attached to the dorsal surface of the cranium using dental cement. Following a 7-14-day recovery period, HTR experiments were carried out in a well-lit room with at least 7 days between sessions to avoid carryover effects. Compounds were dissolved in saline and injected IP. Mice (n = 5–7/group) were injected with drug or vehicle and then HTR activity was recorded in a glass cylinder surrounded by a magnetometer coil for 30 min. Median effective doses (ED50 values) for HTR dose-response experiments were calculated by nonlinear regression.

**Results:** DOB (ED50 = 0.75 μmol/kg; $F(5,27) = 28.79$, $p < 0.0001$) and 2C-B (ED50 = 2.43 μmol/kg; $F(5,25) = 17.60$, $p < 0.0001$) induced the HTR. The benzodifurans DOB-DFLY (ED50 = 0.20 μmol/kg; $F(4,23) = 24.62$, $p < 0.0001$) and 2C-B-DFLY (ED50 = 1.07 μmol/kg; $F(5,28) = 12.88$, $p < 0.0001$) were also active and had significantly higher potency than DOB and 2C-B, respectively. The tetrahydrobenzodifurans DOB-FLY (ED50 = 0.67 μmol/kg; $F(4,21) = 15.61$, $p < 0.0001$) and 2C-B-FLY (ED50 = 1.79 μmol/kg; $F(5,24) = 21.22$, $p < 0.0001$), by contrast, were approximately equipotent with their non-rigid counterparts. Human and mouse data were available for 28 hallucinogens; for those agents, a significant correlation ($r = 0.9682$; $F(1,26) = 389.50$, $p < 0.0001$) exists

between HTR ED50 values in mice and hallucinogenic potencies in humans.

**Conclusions:** The in vivo potency of 2,5-dimethoxyphenylalkylamines is enhanced when the 2- and 5-methoxy groups are incorporated into aromatic furan rings, whereas potency is not altered if the methoxy groups are incorporated into dihydrofuran rings. Potency was also increased in compounds containing an α-methyl group. The unusually high potency of DOB-DFLY is probably linked to the presence of two structural features (a benzodifuran nucleus and an α-methyl group) known to enhance the potency of phenylalkylamine hallucinogens. The potency relationships for these and other compounds in mice closely parallel the human hallucinogenic data. Although head twitches likely have limited value as a model of hallucinogenesis, the HTR assay is useful for investigation of the structure-activity relationships of hallucinogens.

**Keywords:** Hallucinogen, Psychedelic Medicine, LSD, 5-HT2A Receptor, Mouse Models

**Disclosure:** Nothing to disclose.

### T223. Knockdown of the Epigenetic Modifier G9a in the Nucleus Accumbens Reduces Basal Anxiety, Cocaine Self-Administration, and Reinstatement Behaviors

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**Background:** Repeated exposure to drugs of abuse induces lasting epigenetic changes in neurons that can promote addiction. Following chronic cocaine or opioid use, dimethylated lysine 9 on histone 3 (H3K9me2) – a enzymatic process mediated by the histone methyltransferase, G9a – is reduced in nucleus accumbens neurons. Consistent with this observation, chronic drug exposure also produces a decrease in G9a levels; however, the relevance of this reduction to addiction-related behaviors in the drug self-administration (SA) model is unknown. In addition, recent studies suggest that G9a is also involved in anxiety behaviors. We investigated the role of G9a knockdown on both basal anxiety and following cocaine SA.

**Methods:** Experiment One: Cocaine SA and G9a knockdown. Anesthetized rats were infused with a vector containing shRNA against G9a (AAV-shG9a, N = 9) or a scrambled control vector (AAV-shSC, N = 9) into the nucleus accumbens shell (NAcSh) followed by a jugular vein catheterization surgery. Rats self-administered cocaine (0.5 mg/kg/injection) on a fixed ratio schedule 3 h/day for 15 days. Following stabilization of cocaine SA, dose-response testing was performed on both fixed and progressive ratio reinforcement schedules in consecutive order over 2 weeks. Following two weeks of forced abstinence, extinction responding was tested for 5 daily sessions. Next, cue-, cocaine-, and footshock stress-induced reinstatement were assessed. Finally, anxiety behavior was measured using the elevated plus maze (EPM).

Experiment Two: Basal anxiety and G9a knockdown in drug naïve rats. Rats received similar AAV-shG9a (N = 8) or AAV-shSC (N = 7) infusions in NAcSh as described above. Three weeks later, these rats were assessed for basal anxiety levels using EPM and marble burying tests. Locomotor activity was assessed as a control.

These studies were approved by the Institutional Animal Care and Use Committee of UTSW and/or MUSC and were conducted in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals.

**Results:** Experiment One: We found that viral-mediated knockdown of G9a levels reduced H3K9 dimethylation in the NAcSh and decreased both cocaine sensitivity and motivation for the drug. G9a knockdown in the NAcSh also decreased context-elicited cocaine seeking during initial extinction testing, and both cocaine- and stress-induced reinstatement of drug seeking following extinction. Rats also exhibited reduced anxiety-like behavior in the EPM test.

Experiment Two: In drug-naïve rats, G9a knockdown in the NAcSh produced anxiolytic-like effects in both the EPM and marble burying tests, with no effect on locomotion.

**Conclusions:** Together with previously published data showing opposite effects of G9a over-expression in the NAcSh, we conclude that G9a exerts bidirectional control over cocaine sensitivity, cocaine reinforcement and relapse to drug-seeking, along with comorbid anxiety-like behaviors. Thus, reductions in G9a protein and H3K9me2 observed after chronic cocaine administration act as counter-adaptations that limit addiction- and anxiety-related behaviors. The results also suggest that reducing G9a activity in the NAc might have therapeutic value for the treatment of drug addiction and co-morbid anxiety observed in many substance use disorder patients. The reduction of G9a may also reduce anxiety in drug-naïve patients, suggesting a potential target to treat general anxiety disorders. Finally, these data suggest that reducing basal anxiety in patients could reduce future drug-taking and drug-seeking behaviors.

**Keywords:** G9a, Histone Methylation, Reinstatement, Cocaine, Anxiety

**Disclosure:** Nothing to disclose.

### T224. Nuclear Histone Deacetylase 5 Functions During Rat Heroin Self-Administration to Attenuate Future Heroin Seeking Behaviors

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**Background:** Addiction is associated with persistent, maladaptive changes in an individual's behavior, particularly the disproportionate reactivity to drug-associated external and internal cues that act as triggers for relapse. In individuals suffering from substance use disorders, strong memory traces are formed that indelibly link external and internal drug-associated cues with the rewarding/euphoric effects of substances like cocaine and heroin. Drug-induced signaling in striatal medium spiny neurons (MSNs) triggers an epigenetic mechanism involving transient dephosphorylation and partial nuclear accumulation of the activity-dependent chromatin-remodeling enzyme, histone deacetylase 5 (HDAC5). Nuclear HDAC5 in the nucleus accumbens (NAc) limits the development of multiple addiction-related behaviors, including cocaine conditioned place preference and cued reinstatement of cocaine seeking in self-administering animals. However, there remain a number of important unanswered questions: (1) does nuclear HDAC5 limit seeking of other abused drug classes (e.g. heroin) and/or non-drug rewards (e.g. sucrose), (2) when does nuclear HDAC5 act to reduce drug seeking, and (3) in which cell type(s) does nuclear HDAC5 function to suppress drug seeking?

**Methods:** Experiment One: To test the effects of nuclear HDAC5 in the NAc on heroin taking and seeking, we bilaterally infused an adeno-associated virus (AAV2) that expressed a nuclear-localized form of HDAC5 (i.e. HDAC5-3SA) in the adult rat NAc. Following NAc-targeted AAV infusion and recovery, all rats underwent a jugular vein catheterization surgery. The following week rats self-administered heroin until stable. Light and tone cues

accompanied each infusion. Next, rats had seven days of withdrawal until a post-abstinence seeking test (extinction day 1) and were then returned daily until extinction criteria were met. Finally, cue- and heroin-induced reinstatement (0.25 mg/kg, i.p.) were tested.

Experiment Two: To determine the effects of HDAC5-3SA on sucrose pellet SA and extinction-reinstatement, we tested new rats using a similar paradigm as in Expt. One.

Experiment Three: To test whether HDAC5 functions during active drug taking and/or during drug seeking, we infused the AAV-HDAC5 3SA into the NAc after stable heroin SA was established, and then examined extinction and reinstatement behavior as in Expt. One.

Experiment Four: To determine in which MSN cell type(s) HDAC5 limits drug seeking, we generated and expressed a cre-dependent, AAV2-DIO-HDAC5-3SA virus in the NAc of D1-cre or D2-cre BAC transgenic rats (NIDA IRP Transgenic Rat Core). The rats were then run through the same heroin SA paradigms as Expt. One.

These studies were approved by the Institutional Animal Care and Use Committee of MUSC and were conducted in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals.

**Results:** We find that nuclear HDAC5 (3SA) in the NAc produces two distinct effects on heroin SA: (1) it decreases the operant discrimination between the drug-paired and unpaired levers in the early acquisition phase and extinction phase of heroin SA, and (2) it reduces heroin seeking on extinction day 1 (context-induced seeking), after cue presentation, and after heroin-priming. While we observed a similar effect on operant discrimination learning during the acquisition of food SA, there were no effects on extinction or reinstatement of food seeking. In addition, no effects on heroin SA were observed when the AAV-HDAC5-3SA was infused after active, stable heroin SA. Finally, some of these effects on heroin SA are specific to D1R- vs. D2R-MSNs.

**Conclusions:** Similar to the effects of nuclear HDAC5 on cocaine seeking behavior, our data demonstrate that HDAC5 limits post-abstinence drug seeking, and it suppresses reinstatement of both cued and drug-primed heroin seeking. However, natural reward taking and seeking behaviors are not affected. Similar to the effects of reducing Npas4 - an HDAC5 target gene - we also observed clear effects in early operant reinforced learning (heroin and sucrose), indicating an important role in this process. In addition, the ability of nuclear HDAC5 to suppress heroin seeking appears to be dependent on its role during active drug taking, possibly through its epigenetic role to alter chromatin state and gene expression subserving long-term memories linking external and internal cues and drug reward experiences. In addition, some of the effects of nuclear HDAC5 are MSN cell type-specific. These new findings suggest that enhancing HDAC5 nuclear function in the nucleus accumbens during drug taking could be engaging a common epigenetic mechanism, and as such, targeting this mechanism could have therapeutic value for reducing the vulnerability for relapse.

**Keywords:** Heroin Self-Administration, Histone Deacetylase, Reinstatement, D1/D2

**Disclosure:** Nothing to disclose.

### T225. An Ethological Approach to Female Social Defeat Stress and Stress-Escalated Alcohol Drinking in Mice

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**Background:** Women report a greater incidence of pathological drinking following exposure to a moderate or severe stressor compared to men. To provide a preclinical model of stress-escalated drinking in women, we recently developed a novel, female social defeat stress protocol that yields persistently escalated, voluntary alcohol consumption in female C57BL/6 J mice. Unlike other models of continuous, female social defeat stress which rely on significant experimental manipulations used to elicit pathological male aggression toward females, the present social defeat stress model takes an ethological approach by relying on characteristic female-directed female rival aggression.

**Methods:** We have previously shown that male C57BL/6 J (B6) mice exposed to repeated attacks from a dominant, male resident will later consume significantly more alcohol than non-defeated controls, and that CRF-R1 antagonism can selectively reduce stress-escalated drinking. Using female mice, we recently developed a model of social stress that relies on interfemale aggression to evaluate the effects of social defeat stress on alcohol consumption, and to test the effectiveness of CRF-R1 antagonism in reducing stress-escalated drinking. Five days before 7-day continuous social defeat stress (CSDS), intact, nulliparous resident CFW females ($n = 25$) were pair-housed with castrated CFW males ($n = 25$) in large cages with pine shavings. These cages were divided in half with clear, perforated partitions and male-female pairs were housed on one side of the divider. During daily 5-minute confrontations, each CFW male was replaced with a B6 female ($n = 10$). B6 females were then housed opposite the CFW female that defeated them and CFW males were rehoused with the CFW female. B6 females were defeated by and housed with a new CFW female daily. Control B6 females ($n = 10$) were housed opposite male-female CFW pairs but were never exposed to social defeat stress. Ten days after the final defeat, females were tested for social interaction with a novel CFW female. Then, B6 females received continuous access to 20% EtOH and water for ten weeks; beginning in week six, they were treated with weekly intraperitoneal injections of the CRF-R1 antagonist, CP376395 (0-17 mg/kg). For alcohol intake data, two-way repeated measures ANOVA were conducted to detect significant differences between stress and control group drinking, and interactions between stress and CRF-R1 antagonism. In the case of a significant drug effect, Dunnett's tests were used to compare doses of CP376395 to vehicle. Mice were cared for according to the NIH Guide for the Care and Use of Laboratory Animals and procedures were approved by the Institutional Animal Care and Use Committee of Tufts University.

**Results:** Similar to intermale aggression, the majority of resident CFW females attacked the B6 intruder within several seconds. Female aggression occurred in the absence of neuroendocrine manipulations and did not require a prior breeding history. When given continuous access to alcohol, defeated B6 females consistently drank more EtOH than controls ($p < 0.01$), but CSDS did not permanently disrupt estrous cycling or induce social avoidance-like behavior. CP376395 (17.0 mg/kg) significantly reduced alcohol consumption but also suppressed water drinking ($p < 0.05$).

**Conclusions:** As in males, a social stress history increased alcohol consumption in defeated females. While evidence points to CRF/CRF-R1 signaling in male stress-escalated drinking, CP376395 does not selectively diminish female stress-escalated alcohol intake. This female continuous social defeat stress protocol will serve as a productive tool for examining novel pharmacotherapeutic targets for sex-specific effects in models of stress-related psychopathologies. Our ongoing work further characterizes the molecular and behavioral phenotype of socially defeated females and investigates the effects of CSDS on PFC-DRN-VTA-NAc circuitry in the context of social stress-escalated alcohol consumption.

**Keywords:** Social defeat stress, female, Alcohol consumption, CRF-R1, chronic social defeat

**Disclosure:** Nothing to disclose.

### T226. Unpredictable Periods of Withdrawal Between Cocaine Self-Administration Sessions is Associated With Enhanced Motivation for Cocaine: Role for the Orexin/Hypocretin System

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**Background:** Unlike addiction to other drugs of abuse, cocaine addiction is characterized by a pattern of episodic drug use. Experienced users typically report using drug 3-4 days per week with abstinence periods of varying length between binges. This pattern can be due to self-imposed abstinence, or related to issues that are difficult to predict, including drug supply, financial constraints or legal intervention. Despite this, preclinical models of cocaine self-administration typically involve giving animals access to drug on a highly predictable daily schedule 5-7 days per week. Here we tested whether introducing periods of abstinence of unpredictable length between self-administration sessions produces stronger addiction-like endophenotypes. Because we have previously shown that the orexin/hypocretin system is more strongly engaged in individuals with high motivation for cocaine, we also tested the effect of the orexin-1 receptor antagonist SB334867 (SB) on the expression of these endophenotypes.

**Methods:** We used our within-session behavioral economics (BE) procedure (Bentzley et al., 2014) to determine 'baseline' motivation for cocaine in male Sprague-Dawley rats. Next, rats were then given short (ShA; 1 h) or intermittent (IntA; 5 min access every 30 min for 6 h) access to cocaine on either a daily basis (14 sessions over 14 consecutive days) or an episodic basis (14 sessions over 28d, with an unpredictable period of 1-4d between sessions). We also included a daily long access (LgA) control group (6 h continuous access), as this model is the current 'gold standard' approach to modeling addiction in rodents. Rats were then re-assessed for cocaine motivation on the BE task, as well as several other addiction endophenotypes, including compulsive (punished) responding for cocaine, cued reinstatement of extinguished cocaine seeking and withdrawal-induced depression-like behavior. As a gauge of orexin system function, the effect of SB (0, 10, 30 mg/kg; i.p.) on demand and reinstatement behavior was assessed and the number of hypothalamic orexin-expressing neurons was quantified.

**Results:** Rats given daily IntA to cocaine exhibited greater escalation of intake, higher economic demand, higher compulsive responding, and higher depression-like behavior compared to rats given daily ShA or LgA to cocaine. The IntA-induced multiphenotype was exaggerated even further when self-administration sessions were separated by unpredictable periods of abstinence (episodic access). In contrast, episodic access did not affect the expression of addiction endophenotypes in ShA animals. SB was effective at reducing motivation for cocaine and cued reinstatement across all groups, but to a greater extent in episodic-IntA rats. In daily- and episodic-IntA rats, a low dose of SB (previously shown not to affect general reward seeking) completely reversed the addiction phenotype. The number of orexin-expressing neurons was higher in IntA rats compared to ShA rats.

**Conclusions:** These data indicate that IntA and episodic self-administration patterns interact to produce an exaggerated addiction-like phenotype, which is hyper-reliant on signaling at the orexin-1 receptor. This might be due to an increase in the number of orexin-expressing neurons. These data are timely given recent speculation regarding the potential repurposing of the FDA-approved suvorexant compound for the treatment of addiction.

**Keywords:** Orexin Receptor Antagonist, Cocaine Self-Administration and Reinstatement, Hypocretin, Animal Models, Withdrawal

**Disclosure:** Nothing to disclose.

### T227. Genetic Factors Disrupt Pregnancy's Protective Effect on Voluntary Alcohol Consumption in Mice Development of a Preclinical Model of Pregnancy-Associated Resilience to Addictive Processes

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**Background:** While pregnancy exerts a more robust and universal temporary protective effect on addictive substance use in women than any intervention to date (1), underlying mechanisms are not clearly elucidated. Early evidence from clinical and preclinical studies suggest that this phenomenon, or pregnancy-associated resilience (PAR), may be an evolutionarily-conserved adaptation facilitated by substrates of maternal behavior (2). Specification of these substrates will inform the development of novel and more effective pharmacologic and behavioral interventions for substance use disorders. Within this context, we describe a preclinical model of PAR for future bidirectional translational research.

Among the substances of abuse for which preclinical models exist, only alcohol closely mimics consumption patterns in humans, and is thus ideally suited for our unique focus on patterns of maternal consumption, rather than the conventional focus on offspring outcomes. Our first aim was to confirm the presence of PAR in alcohol-preferring (C57BL) mice, (observed to consume alcohol at levels comparable to moderate drinking in humans), as evidenced by reduction in voluntary alcohol consumption in pregnant dams, from conception through the early postpartum period, but not in non-pregnant female controls across the same period of time.

Clinically, drinking behavior in patients with alcohol use disorders is influenced by both pre-existing heritable influences (3) and neural adaptations related to chronic alcohol use—influences that are challenging to disentangle (4). In C57BL mice, pre-pregnancy alcohol experience appears to attenuate, but not extinguish PAR (5). The impact of heritable influences, independent of pre-pregnancy alcohol-related processes has translational relevance but has not been studied to our knowledge. Discerning the relative contribution of family history of alcoholism, versus women's pre-pregnancy patterns of alcohol use, for example, would enable obstetricians to personalize treatment based on these patient characteristics. Thus, in the second aim, we examined the impact of heritable risk on PAR by replicating the above experiment in a strain of high alcohol-preferring (HAP) mice, specifically bred for heavy alcohol consumption (6).

**Methods:** All procedures were approved by the University Institutional Review Board and conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Sixteen virgin female C57BL dams were housed individually in polycarbonate cages with micro-isolator tops, exposed to 12-h light/12-h dark cycles, and allowed free access to 10% alcohol (v/v in distilled water), distilled water, and chow, consistent with an established voluntary consumption paradigm (7). After one week, dams were allowed to mate with a male C57BL mouse, then returned to separate cages. Alcohol consumption was quantified,

as percentage of total fluid intake, prior to mating, after each trimester of pregnancy, and one week postpartum. Mean within-group differences in percentage alcohol consumption at each measurement interval were assessed with repeated measures ANOVA. This protocol was repeated in HAP mice.

**Results:** In C57BL mice, we observed the expected reduction in alcohol consumption among pregnant dams ($n = 10$) from conception (5.7%) through one week postpartum (3.0%; mean diff = 2.724; 95% C.I. [0.6 – 4.8]; $p = .0125$), but not in non-pregnant C57BL controls ($n = 6$; 5.6% to 5.9%; $p = 0.51$). In HAP mice, neither pregnant ($n = 3$) nor control dams ($n = 17$) reduced alcohol consumption. Due to the small number of pregnancies in HAP dams, the HAP experiment is being repeated at present. Final results will be presented.

**Conclusions:** Evidence of PAR in C57BL mice confirms PAR as an evolutionarily-conserved adaptation. Absence of PAR in HAP mice suggests that PAR may be more susceptible to heritable risk than to alcohol-related processes. This finding, if confirmed, has translational relevance for personalizing prenatal preventive interventions. Specifically, family history of alcoholism could be a better predictor of difficulty cutting down or abstaining from alcohol across gestation than pre-pregnancy drinking patterns.

**Keywords:** Alcohol and Substance Use Disorders, Animal Models, Pregnancy, Risk and Resilience

**Disclosure:** Nothing to disclose.

### T228. Neural Circuit Mechanisms of Stress-Induced Alcohol Seeking Behavior

Abstract not included.

### T229. Relapse Depends on the Type of Cue and the Type of Brain: Pavlovian Cues Versus Occasion Setters, Sign- Versus Goal Trackers, Dopamine Versus Acetylcholine

Abstract not included.

### T230. Viral-Mediated Knockdown of ARC in the Nucleus Accumbens Alters Novelty, Mood, and Drug-Related Behaviors

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**Background:** Glutamatergic plasticity, particularly changes in the expression, localization, and function of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), is a common mechanism in multiple forms of experience-dependent plasticity. Preclinical models of learning and memory, drug addiction, and mood disorder have all identified forms of AMPAR plasticity in a variety of brain regions, and many groups have demonstrated behavioral relevance for this plasticity. One key locus for experience-dependent changes in AMPAR plasticity is the nucleus accumbens (NAc). The immediate early effector gene, activity-regulated cytoskeleton-associated protein (Arc, also known as Arg3.1), is a known regulator of AMPAR surface expression, both through the promotion of endocytosis and the regulation of GluA1 expression. Arc mRNA is upregulated by stress-experience and acute cocaine exposure in the NAc and given that AMPAR regulation is a key site of experience-dependent glutamatergic plasticity, Arc has potential to be a molecular contributor to this plasticity. In our initial observations of Arc-deficient mice (Arc KO), we found enhanced cocaine locomotor sensitization, enhanced cocaine conditioned place preference following repeated cocaine administration, reduced social novelty detection, and reduced anxiety- and depression-like phenotypes. Using a viral-mediated rescue strategy in Arc KO mice, we previously implicated the NAc in mediating some of the anxiety and drug-related phenotypes, but it remains unclear which phenotypes are developmentally derived, and which are dependent on Arc's function in the adult brain.

**Methods:** To address the role of Arc in the adult NAc, we used a viral-mediated shRNA knockdown approach followed by behavioral testing. Young adult male and female mice received either AAV2-shArc or AAV2-shControl virus bilaterally in the NAc. We performed two different studies: (1) to maximal reduction of Arc levels, we compared AAV2-shArc vs. AAV2-shControl in mutant mice lacking one copy of the Arc gene (Arc + /GFP-KI or "Arc hets"), and (2) to test effects in WT mice, we compared AAV2-shArc vs. AAV2-shControl in C57BL/6 J WT mice. Following surgical recovery and viral-mediated Arc knockdown, all mice were examined for performance on social-, novelty-, drug-, and mood-related behaviors.

**Results:** We find that shArc effectively reduces Arc expression in heterologous cells, primary neurons, and in vivo. Arc hets perform similarly to wild-type mice in the examined behavioral tests, but the Arc hets have the benefit of genetically-reduced Arc expression, which is further reduced by AAV2-shArc in the NAc. Preliminary findings in Arc hets demonstrate that NAc-restricted knockdown of Arc (shArcNAc) appears sufficient to reduce anxiety and enhance cocaine reward, with no effect on cocaine locomotor sensitization. To examine possible developmental effects of genetically-reduced Arc (Arc hets) that might influence the AAV2-shArcNAc effect on these behaviors, we again examined a number of behaviors in wild-type (WT) mice using AAV2-shArc in the adult NAc. Interestingly, AAV2-shArcNAc in WT mice was not sufficient to reduce anxiety- or depression-like behavior, but it did produce reduced social novelty detection. Additionally, AAV2-shArcNAc in WT mice disrupted novel object recognition.

**Conclusions:** Overall, our results suggest that Arc in the NAc regulates some, but not all, of the behavioral phenotypes observed in the global Arc KOs. This indicates that some Arc KO behaviors are produced by Arc's function in other brain regions and/or developmental roles in the brain. Interestingly, we observed strong behavioral consequences of AAV2-shArcNAc of Arc hets, which could be due to the greater extent of Arc reduction when combined with Arc gene copy reduction and/or to subtle subthreshold developmental effects. Surprisingly, AAV2-shArcNAc in WT mice did not alter anxiety- and depression-like behaviors, but we did observe deficits in novel social and object detection and/or preference, without influencing sociability or exploratory behaviors. Together, our findings suggest an essential role for Arc, specifically in the NAc, in novelty, reward and/or salience behavior. Current work is focused on examining the electrophysiological consequences of AAV2-shArcNAc and generating new reagents to enable cell type-specific manipulation of Arc levels.

**Keywords:** Drug Addiction, Molecular Mechanisms, Activity-Regulated Cytoskeletal Associated Protein (Arc), Nucleus Accumbens

**Disclosure:** Nothing to disclose.

### T231. Pharmacological Properties of the Type-2 alpha Corticotrophin Releasing Factor Receptor/Dopamine D1 Receptor Heteromer

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**Background:** Dopamine and corticotrophin releasing factor (CRF) are neurotransmitters implicated in stress-related addictive behavior. Previous work has shown that CRF2α and D1R are able to heteromerize in living cells (Fuenzalida, Galaz et al., 2014). Thus, we decided to study the consequences of CRF2α/D1R heteromerization in the signaling and localization of both receptors.

**Methods:** To this end we used over-expression of CRF2α and D1R in HEK293T cells and rat prefrontal cortex synaptosomes.

**Results:** The presence of dopamine in the incubation medium significantly decreased the amount of CRF2α on the cell surface of HEK293T cells expressing both receptors. We also observed that the presence of both receptor agonists increased the interaction between CRF2α and D1R. The activation of mitogen-activated protein kinase/extracellular signal-regulated kinase (ERK) by each agonist alone was significantly decreased when the cells co-expressing both receptors were co-incubated with dopamine and CRF. This change in the signaling was also observed using rat prefrontal cortex synaptosomes.

**Conclusions:** These results provide new evidence of the functional consequences of the heteromerization between CRF2α and D1R. Further studies should address whether the changes in signaling and localization of CRF2α and D1R due to their heteromerization are involved in stress-related addictive behavior.

**Keywords:** D1 Dopamine Receptors, Corticotropin-Releasing Factor (CRF), Receptor Heteromerization

**Disclosure:** Nothing to disclose.

### T232. A Dynorphin Projection From the Dorsal Raphe Nucleus to the Ventral Tegmental Area Mediates Stress Potentiated Cocaine Reward in Mice

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**Background:** Stress potently enhances the development, maintenance, and relapse of addictive behaviors. Dynorphin, the endogenous kappa opioid receptor (KOR) ligand, encodes dysphoric states associated with stressful stimuli, and chronic exposure to drugs of abuse increases dynorphin mRNA expression in humans. Dynorphin/KOR actions on dopamine neurons have been shown to underlie aversive learning, and we hypothesized that potentiation of cocaine reward following stress is likely to occur through similar neural mechanisms. The studies presented here investigate the neural substrates underlying stress-mediated enhancement of cocaine reward.

**Methods:** A CAV2 vector with Cre-dependent expression of a green fluorophore (Zs-Green) was used in prodynorphin (pdyn)-Cre mice to identify dynorphin projections into the ventral tegmental area (VTA). Our behavioral experiments used a cocaine conditioned place preference (CPP) procedure. Male and female C57BL/6 mice were exposed to repeated forced swim stress, optogenetic inhibition, or were gently handled prior to conditioning sessions. Optical stimulation occurred with Channelrhodopsin-2 (ChR2), and optical inhibition occurred with Step Waveform inhibitory Channelrhodopsin (SwiChR). Floxed dynorphin, KOR, or p38α MAPK mice were used for cell-specific and regional deletion of the dynorphin/KOR system or downstream signaling substrates. Fiber photometry was used in DAT-Cre mice injected with GCaMP6m in the VTA to determine the effect of KOR activation on calcium activity in dopamine neurons during behavioral testing.

**Results:** Dynorphin-containing neurons projecting to the VTA were identified by injection of CAV2-DIO-ZsGreen into the VTA of pdyn-Cre mice. High densities of VTA-projecting dynorphin neurons were observed in the dorsal raphe nucleus (DRN) and prefrontal cortex. Optical stimulation of dynorphin neurons in the DRN (pdyn-Cre mice with ChR2) produced KOR phosphorylation in the ventral tegmental area (VTA) that could be blocked by pre-treatment with a KOR antagonist. Immunohistochemical analyses showed that KORs were expressed in a majority of dopamine neurons in the medial and lateral VTA. Male and female C57BL/6 mice demonstrated potentiation of cocaine CPP following repeated forced swim stress (rFSS), which has been previously shown to be KOR-dependent. Optical stimulation of ventral tegmental area (VTA) dopamine neurons (DAT-Cre) produced CPP that could be potentiated by rFSS, indicating that optically-evoked reward learning could also be potentiated by KOR activation. Deletion of dynorphin from the DRN or conditional deletion of KORs from dopamine neurons prevented swim stress-induced enhancement of cocaine CPP. Cocaine CPP potentiation is likely to be arrestin-dependent, as conditional deletion of p38α MAPK from dopamine neurons also prevented stress-induced potentiation of cocaine CPP. Using in vivo fiber photometry in DAT-Cre mice expressing GCaMP6m, we found that KOR activation increased calcium activity in dopamine neurons. Current experiments aim to determine whether increased calcium mobilization requires KOR/p38 activation in dopamine neurons. KOR activation is also known to inhibit electrophysiological activity of dopamine neurons, and we modeled the effect of KOR activation on cocaine CPP potentiation using SwiChR. DAT-Cre mice received a 30-min session of optical inhibition with SwiChR prior to or during cocaine conditioning. Similar to effects previously observed with KOR activation, optical inhibition prior to conditioning potentiated cocaine CPP, whereas concurrent inhibition of dopamine neurons with cocaine treatment ablated cocaine preference.

**Conclusions:** Our studies show that dynorphin neurons in the DRN are functionally connected to the VTA and required for stress-induced increases in cocaine reward. Although striatal dynorphin is known to modulate activity of dopamine neuron terminals, the source of dynorphin for somatodendritic KORs in the VTA was previously unknown. We found that dynorphin release from the DRN into the VTA activates KOR and enhances cocaine reward in an arrestin-dependent manner. Activation of the p38 MAPK is hypothesized to lead to long-lasting changes in the excitability of dopamine neurons, altering reward processing after stress and increasing reward-seeking behaviors. Future studies will aim to determine the specific signaling pathways involved in these changes in excitability and neural circuits underlying these effects.

**Keywords:** Kappa Opioid Receptor, Cocaine, Dynorphin, Acute Stress

**Disclosure:** Nothing to disclose.

### T233. HDAC5 Differentially Regulates Cocaine Self-Administration and Seeking Behavior in the Prelimbic and Infralimbic Cortices

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**Background:** Relapse to drug seeking remains a major obstacle in the treatment of cocaine addiction in individuals suffering from substance use disorders. Underlying this clinical obstacle are

neuroadaptations and changes in gene expression within limbic structures critical for reward and goal-directed behavior. Drug-induced changes in gene expression in the medial prefrontal cortex (mPFC) have been shown to promote drug-seeking or to develop as a compensatory mechanism to oppose drug-reward. These changes can occur through epigenetic mechanisms such as histone acetylation and deacetylation, which changes chromatin structure through post-translational modification of histone N-terminal "tails", altering the accessibility of genomic DNA at specific genomic regions. Histone deacetylase 5 (HDAC5) is a class IIa HDAC that is dynamically shuttled in and out of the nucleus in an activity-dependent manner. Its cellular localization is regulated by phosphorylation of conserved residues in and around its nuclear localization sequence. We have previously shown that cocaine causes a transient nuclear localization of HDAC5 in nucleus accumbens (NAc) medium spiny neurons (MSNs) and overexpression of a nuclear-localized HDAC5 in the NAc reduces both cue- and primed-induced drug seeking after cocaine self-administration. However, the role and regulation of HDAC5 in addiction-relevant NAc efferents, such as the mPFC, has not been investigated. The mPFC is divided into distinct subcortices including the prelimbic and infralimbic cortices (PrL and IL) that have distinct and often opposing functions in both substance use and stress disorders.

**Methods:** Here we tested the hypothesis that viral-mediated nuclear HDAC5 (HDAC5 S259A/S279A/S498A or "3SA") expression in the PrL cortex, a region associated with the promotion of drug-seeking behavior, would decrease cocaine-seeking behavior, but possibly have a functionally opposing role in the IL. We delivered adeno-associated virus expressing HDAC5 3SA or a control into either the PrL or IL prior to cocaine self-administration, and assayed behavior in an extinction-reinstatement paradigm. Because manipulations of the PrL and IL have been shown to regulate stress and fear responses, we also assayed the effect of HDAC5 3SA expression in these regions on stress-induced reinstatement and anxiety behavior. Finally, we investigated how HDAC5's subcellular localization is regulated by cocaine self-administration in PFC pyramidal neurons.

**Results:** We found that expression of HDAC5 3SA in the PrL increased active cocaine self-administration behavior and expression in IL showed the opposite effect. Furthermore, HDAC5 expression suppressed drug-seeking behavior in both regions, but in a modality specific manner. HDAC5 3SA expression in the PrL suppressed context-induced drug seeking, while expression in the IL appears to reduce cocaine-primed reinstatement of drug seeking. Our preliminary findings suggest that nuclear HDAC5 in the PrL, but not the IL, might increase stress-induced drug-seeking and anxiety-like behavior. We also find that HDAC5 is regulated by activity and cAMP signaling in a similar manner to striatal neurons, and ongoing studies are investigating HDAC5's subcellular localization in vivo following cocaine self-administration in a region, layer, and cell-type specific manner.

**Conclusions:** Our work is consistent with previous studies implicating a role for both epigenetic alterations in the mPFC in cocaine self-administration and seeking, and a role for HDAC5 in substance abuse behavior. However, our data demonstrates that HDAC5's regulation of cocaine self-administration behavior is regionally specific and divergent. Because HDAC5 appears to have opposing functions in the PrL and IL, future studies will investigate if HDAC5 is acting differentially within these subregions or through opposing circuits to differentially modulate synapse strength within drug-seeking circuits and to determine both when and how nuclear HDAC5 modulates drug seeking behaviors in the mPFC.

**Keywords:** Addiction Circuitry, Molecular Mechanisms, Intravenous Drug Self-Administration, Epigenetics

**Disclosure:** Nothing to disclose.

## T234. EZH2 is an Epigenetic Regulator of Molecular Changes in the Amygdala Caused by Adolescent Alcohol Exposure in Humans and Rodents

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**Background:** Drinking during adolescence is a primary risk factor for developing an alcohol use disorder and comorbid mental problems (i.e. anxiety) later in life. Recent work has demonstrated that epigenetic factors play an important role in the amygdala, a critical brain region for both adolescent brain development, alcohol abuse disorders, and anxiety. Accumulating evidence indicates that disruption of brain derived neurotrophic factor (BDNF) signaling in the amygdala by alcohol produces abnormal synaptic plasticity and contributes to anxiety and alcohol drinking behaviors in rodents, however this has yet to be demonstrated in humans. Additionally, the epigenetic mechanisms that contribute to altered BDNF signaling are still poorly understood. This study investigated how adolescent drinking effects BDNF signaling in human postmortem and rat amygdala and related epigenetic mechanisms mediated by EZH2 (histone methyl transferase) component of polycomb repressive complex 2 (PRC2) complex.

**Methods:** Human postmortem amygdala was acquired from individuals with AUD who began drinking before and after the age of 21 (New South Wales Brain Tissue Resource Center, Sydney, Australia) and was subjected to qPCR and chromatin immunoprecipitation (ChIP) assay. To study the role of EZH2, Sprague Dawley male rats were exposed with intermittent injections (2 days on and off schedule) of ethanol (2 g/kg) or saline during adolescence (postnatal days 28-41) then allowed to mature to adulthood. Rats were then infused with an siRNA targeting EZH2 into the CeA then evaluated for anxiety-like behaviors and epigenetic modifications.

**Results:** Studies in human postmortem amygdala revealed that individuals who began drinking before the age of 21 had decreased BDNF mRNA and protein expression but not in individuals who began drinking after the age of 21. ChIP analysis revealed that this decrease was likely caused by increased repressive H3K27me3 deposited by increased EZH2 at BDNF-IX promoter in individuals who began drinking before but not after the age of 21. Correlation analysis revealed that decreases in BDNF expression was not correlated with number of drinking years suggesting that this effect may be more specific to the developmental period when drinking began. In order to determine if EZH2 is involved in epigenetic regulation, we utilized a reverse translational rat model of adolescent alcohol exposure. Our results indicate that exposure to alcohol during adolescence caused decreased Bdnf expression, increased anxiety, increased EZH2 and H3K27me3 associated with the BDNF-IX promoter, and that this can be prevented by knocking down EZH2 in the CeA of rats in adulthood. We next evaluated changes in a downstream target of BDNF signaling, activity-regulated cytoskeleton-associated protein (ARC) in human postmortem brain. Similar to BDNF expression, ARC expression was decreased in individuals who began drinking before the age of 21 but not after the age of 21. ChIP analysis revealed increased H3K27me3 and EZH2 at the ARC synaptic activity response site (SARE). EZH2 knockdown in the CeA of adult rats exposed to alcohol during adolescence prevented decreases in Arc mRNA expression and increases in H3K27me3 and EZH2 associated with the Arc SARE site.

**Conclusions:** This data suggests that EZH2 is a conserved epigenetic mediator of changes in adulthood due to adolescent

alcohol drinking and could be a potential target for treatment of alcohol use disorders that begin during adolescence. (Supported by NIH-NIAAA P50AA022538, UO1AA019971, U24AA024605, RO1AA010005 and by the VA Senior Research Career Scientist award to SCP).

**Keywords:** Amygdala, Epigenetics, Adolescent Alcohol, Anxiety, BDNF

**Disclosure:** Nothing to disclose.

### T235. Weighted Transcriptome Analysis Identifies Network Module in Hippocampus Chronically Exposed to Cocaine

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**Background:** The hippocampus is a brain region involved in addiction and drug-associated learning and memory. In the hippocampus, long-term adaptation requires chromosome remodeling and gene expression changes. Several recent studies have shown that mitochondria play an important role in dendritic arborization and spine formation. Previously, we found that mitochondrial function associated genes are significantly downregulated in the hippocampus of chronic cocaine addicts. Here, we have performed machine learning based netwok analysis to predict the gene regulatory networks that are altered in brains that have been chronically exposed to cocaine.

**Methods:** Eight chronic cocaine addicts, six cocaine addicts with excited delirium, eight alcoholics, and eight drug-free control subjects were selected for the study. Each hippocampal tissue sample was carefully matched between treatment groups for age, ethnicity, and postmortem interval and RNA quality as previously reported (Zhou et al, 2011) in detail. Using the Ion Torrent next generation sequencing technology, we examined gene expression changes in the transcriptome of these patients. The weighted gene co-expression network analysis (WGCNA) was performed and identified significantly enriched network modules. We performed gene ontology annotation and KEGG pathway enrichment analyses for these modules to identify their putative functions.

**Results:** Among the 25 color modules identified by WGCNA alogorithm with an adjusted p value less than 0.05, dark magenta and turquoise modules were significantly enriched across cocaine, cocaine with delirium, and alcohol exposed brains. We subjected the genes within these color modules to Gene Ontology (GO) and pathway analysis to detect molecular and cellular functional domains impacted by chronic cocaine exposure, cocaine exposure with delirium, and alcohol dependence. We found that these significant colored modules contained genes involved in mitochondrial inner membrane functions, oxidative phosphorylation, mitochondrial genes responsible for ATP synthesis, electron transport, maintenance of mitochondrial membrane potential, and defense against oxidative stress. In fact, further analysis revealed that genes that were previously identified as statistically significant in transcriptome data were even more highly enriched in the Gene Ontology WGCNA analysis.

**Conclusions:** Using whole transcriptome and WGCNA analysis, we identified a novel hippocampal network module in cocaine-addicted, cocaine-addicted with excited delirium, and alcoholics. We observed genes in the module that were related to decrease mitochondrial inner membrane functions, oxidative phosphorylation, and energy metabolism. Interestingly, these mitochondrial changes are also observed in neurodegenerative diseases such as Alzheimer and Parkinson's disease. These results indicate this organelle may play an important role in long-lasting maladaptation occurring in the substance abuse and addiction, and possibly dysregulated mitochondrial function is of critical importance in a subset of addicted cocaine users.

**Keywords:** Cocaine Addiction, Alcohol Dependence, Network-Analysis, Mitochondria, RNA Sequencing

**Disclosure:** Nothing to disclose.

### T236. Gβγ Subunits Released During GPCR Activation Bind to the Dopamine Transporter and Facilitate an Efflux Mode of the Carrier

Abstract not included.

### T237. Capturing Habitualness of Drinking and Smoking Behavior in Humans

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**Background:** There is a great deal of interest in elucidating habit driven behavior, particularly in the context of addiction. Habitual behaviors are characterized by rigid, inflexible actions, which are triggered by associated stimuli, and are performed despite their immediate consequence; whereas goal-directed behaviors are performed in order to achieve an expected outcome. The neuroscience of addiction has suggested distinct neural substrates for goal directed versus habitual alcohol and drug intake. While these insights are compelling at the preclinical level of analysis, they clearly necessitate a translation to clinical samples. The scientific premise of this study is that examining patterns of alcohol and cigarette use may offer valuable insights into the habitualness of these behaviors.

**Methods:** A community sample of treatment-seeking heavy drinking daily smokers (N = 279, 34% female, average age of 43.6 years old) completed an in-person diagnostic and clinical evaluation battery. Assessments included the Timeline Follow Back (TLFB) for both alcohol and cigarette consumption over the past 30 days. All participants had a breath alcohol concentration of 0.00 g/dl and tested negative for drugs of abuse at the time of the evaluation. The Habit Index Form was completed separately for smoking and drinking behaviors. Interclass correlation coefficients (ICCs) with agreement were calculated for both smoking and drinking based on the past 30-day TLFB data with observations being nested within days of the week (1-7) and weeks (1-4) thus quantitatively capturing the degree to which participants drug use was consistent week over week during the four-week period assessed (higher ICC = more consistent use pattern).

**Results:** Regression models were used to examine whether a stronger pattern of drinking and smoking were associated with self-reported habit, age, sex, and severity of alcohol and nicotine use disorder. The average ICC for this sample was higher for smoking (Mean = 0.35, SD = 0.41) than for drinking (Mean = 0.32, SD = 0.33). There was a significant correlation between the ICC for smoking and the ICC for drinking ($r = 0.39$, $p < .001$). Age was positively associated with smoking ICC ($r = 0.23$, $p < .001$), such that older smokers reported higher smoking ICC. There was no effect of age on the ICC for drinking ($p = .40$) nor was there a sex effect for either smoking ($p = 0.60$) or drinking ($p = 0.59$) ICCs. Analyses examining the patterness (i.e., indexed by ICCs) of smoking and drinking behavior in relation to scores on the habit index are underway.

**Conclusions:** This study seeks to characterize habitualness of smoking and drinking behaviors through analyses of their consistency, or patterness, over time. Initial results suggest that the degree of consistency in smoking and drinking in associated with one another in this sample of alcohol and cigarette co-users. There was no evidence of sex-effects and older individuals reported more "patterned" smoking behavior. Further analyses are underway to validate the approach of using pattern to index habit. The overarching goal of this research is to obtain a clinical useful index of habitualness of smoking and drinking that can be used to translate preclinical findings.

**Keywords:** Habit, Alcohol, Smoking

**Disclosure:** Nothing to disclose.

### T238. Investigating the Relationship Between Immune Functioning Markers and Cognition in Cocaine Use Disorder: A LASSO Approach

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**Background:** Cocaine use impairs cognition, and such impairments reduce the efficacy of cognitively-demanding treatments such as Cognitive-Behavioral Therapy. Identifying factors that produce these impairments is thus important to reducing cocaine use. Previous research suggests drug use increases inflammatory immune markers, particularly TNFα and IL-6 cytokines. Inflammation is linked to cognitive impairment in other psychiatric disorders. However, the link between inflammation and cognition in cocaine users has been only minimally investigated.

**Methods:** Data was collected from 90 cocaine users aged 18-60, during screenings for studies of Cocaine Use Disorder. The final composite score of the Shipley-2, a brief measure of crystallized and fluid cognitive ability, was our measure of cognition. As part of another project, blood samples were tested for 29 immune markers using Bio-Plex Pro Human Inflammation Panel Assays. We utilized least absolute shrinkage and selection operator (LASSO) regression as a principled approach to identifying which baseline characteristics (i.e. age, gender) and immune markers related to cognition. Individual regressions were then used to describe the relationship between immune markers identified by the LASSO and Shipley scores while controlling for the baseline characteristics identified by the LASSO.

**Results:** Eighteen immune markers produced sufficient detectable levels for analysis. The LASSO identified gender, Eotaxin-2, and TWEAK as the most important predictors of Shipley score. After controlling for gender, Eotaxin-2 ($B = 5.27$, SE $B = 1.45$, $t = 3.640$, $p = < 0.001$) and TWEAK ($B = 3.03$, SE $B = 1.46$, $t = 2.072$, $p = 0.04$) levels positively related to Shipley scores.

**Conclusions:** Unexpectedly, higher levels of Eotaxin-2 and TWEAK related to better cognitive performance. These results are surprising as these are typically considered inflammatory markers. Limitations include the use of a cross-sectional sample of convenience, and omission of some cytokines often implicated in psychiatric disorders (e.g. IL-6, IL-10). Nevertheless, our results suggest a more nuanced relationship between immune functioning and cognition in Cocaine Use Disorder, such that some immune activation may have neuroprotective properties.

**Keywords:** Cocaine Addiction, Cognition, Immune Biomarkers

**Disclosure:** Nothing to disclose.

### T239. Acute Stress Effects on Decision-Making in Gambling Disorder

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**Background:** Given the clinical relevance of both stress and risk-taking behaviours in gambling disorder (GD), the current study investigates the effects of stress and how these might predict different aspects of behavioural control. In particular, risk-taking and decision-making are relevant features in GD. Risk-taking behaviours and stress both activate the sympathetic nervous system and are implicated in addiction processes. Nonetheless, mechanisms underlying the relationship between the stress response and engagement in risky behaviours are not well understood. While alterations in stress system reactivity have been observed in GD populations few studies exist characterizing responses to stress and how these might relate to risk-taking in GD. The current study integrates multiple indices of stress, incorporating physiological, subjective and behavioural measures. Given the close association between stress and GD, a study of the relationship between stress reactivity and risk-taking provides an important link between biological processes and risky behaviour.

**Methods:** Thirty-eight participants who met criteria for GD and 28 healthy control (HC) participants were randomized to a stress or a no-stress condition. The stress group underwent the Trier Social Stress Test, a validated measure of acute psychosocial stress. All participants completed two validated measures of risky decision-making: the Balloon Analogue Risk Task (BART) and the Iowa Gambling Task (IGT). Saliva samples as well as measures of mood and gambling urges were collected at baseline, post-stressor and post-task. Saliva samples were subsequently assayed for levels of the hormone cortisol.

**Results:** Stress increased cortisol levels in both HC and GD groups, but there were no between-group differences in cortisol. While stress increased subjective reports of mood disturbance in the HC group, the stressed GD group did not report a significant rise in mood disturbance following the stressor. In the GD group, gambling urges did not differ between stressed and non-stressed participants, however, after completing the IGT and BART (i.e. post-task), the non-stressed GD group reported a significant increase in gambling urges. There were no group or stress effects on IGT score. However, following stress, the GD group made significantly less risky choices on the BART, compared to the non-stressed GD group. The stressed GD group also showed an inverse relationship between IGT performance and BART score. There were no significant correlations between cortisol with subjective or behavioural measures.

**Conclusions:** The current study examined the effects of acute stress on risky decision-making and found several differential effects of stress in a GD population. The stressed GD group demonstrated a heightened cortisol response following the stressor but did not report a significant increase in mood disturbance. This finding suggests a difference between stress reactivity and stress appraisal in this group and is consistent with the idea of interoceptive awareness alterations in GD. Unexpectedly, acute stress also produced a significant reduction in risk-taking on the BART in the GD group, suggesting that acute stress may have increased this group's sensitivity to negative consequences on the task. These results will be discussed in the framework of current knowledge of risky decision-making in GD.

**Keywords:** Risky Decision-Making, Acute Stress, Gambling Disorder

**Disclosure:** Nothing to disclose.

### T240. Sensitivity to Uncertain Threat is Exaggerated in Individuals With Alcohol Use Disorder and Associated With Their Drinking Behaviors and Motives

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**Background:** Emerging research suggests that individuals with alcohol use disorder (AUD) display exaggerated anticipatory anxiety in response to uncertain threats or stressors (U-threat). Accordingly, a developing theory is that individuals who are chronically hyper-reactive to U-threat find alcohol intoxication to be especially reinforcing, which sets the stage for negative reinforcement processes to drive excessive alcohol use. Although there is promising initial behavioral evidence to support this theory, several important questions remain. Most notably, little is known about the neural bases that underlie reactivity to U-threat; although the anxiety disorder literature suggests that the anterior insula (aINS) and dorsal anterior cingulate cortex (dACC) are two key nodes involved in generating anticipatory anxiety. In addition, the extent to which behavioral and neural reactivity to U-threat map onto the severity of AUD illness as well as negative reinforcement processes such as drinking to cope with negative affect is unknown.

**Methods:** The current study tested three related questions and had three aims: 1) Consistent with prior studies, do individuals with AUD display exaggerated behavioral reactivity to U-threat measured via startle eyeblink potentiation; 2) Do individuals with AUD display exaggerated neural reactivity to U-threat measured via functional magnetic resonance imaging (fMRI); 3) Do behavioral and brain responses to U-threat correlate with AUD symptom severity and coping motivated drinking? Participants included adult volunteers ($n = 60$) with and without a lifetime history of AUD (36 AUD and 24 non-AUD). All individuals completed a well-validated threat-of-shock task designed to probe responses to U-threat and predictable threat (P-threat) while startle eyeblink potentiation was collected as an index of aversive responding. Individuals also completed a newly-designed, analogous fMRI version of the threat-of-shock task inside the scanner. All individuals additionally completed the Alcohol Use Disorders Identification Test (AUDIT), a widely used self-report measure of AUD symptoms, and the Drinking Motives Questionnaire-Revised (DMQ-R).

**Results:** For Aim 1, results indicated that individuals with AUD displayed greater startle eyeblink potentiation during U-threat ($F[1,59] = 4.44$, $p < .05$), but not P-threat ($F[1,59] = 1.16$, $p = .21$), compared with individuals without AUD. For Aim 2, individuals with AUD displayed greater bilateral anterior insula activation (anatomical aINS region-of-interest [ROI] analysis; $F[1,59] = 3.93$, $p < .05$) during U-threat. Lastly, for Aim 3, results revealed that both startle eyeblink and aINS response to U-threat were positively correlated with self-reported AUDIT scores (startle: $r = .28$, $p < .05$; aINS: $r = .36$, $p < .05$) and coping motives for alcohol use (startle: $r = .30$, $p < .05$; aINS: $r = .26$, $p < .05$).

**Conclusions:** Results indicate that individuals with AUD display exaggerated sensitivity to U-threat at the behavioral and neural level. The study demonstrates for the first time that individuals with AUD display increased aINS reactivity during U-threat – a key region in the brain known to mediate anxious responding. The findings also reveal that these multimethod biomarkers track the severity of AUD illness and tap into drinking behaviors that are motivated by negative reinforcement processes such as coping with negative affect. Together, these findings point to exaggerated reactivity to U-threat as a novel phenotype for AUD prevention and/or intervention.

**Keywords:** Threat of Shock, Functional MRI (fMRI), acoustic startle response, alcohol use disorder

**Disclosure:** Nothing to disclose.

### T241. Connecting Symptomatology to Impulsive Decision-Making in Opioid Use Disorder Through Psychophysiological Measures

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**Background:** Impulsive decision-making is characteristic of addictive disorders and is associated to changes in the brain valuation network due to chronic substance abuse. Computations in this network have been shown to be strongly context-dependent and to explain variation in decision-making behavior in healthy subjects. We hypothesized that changes in clinical states — particularly high-craving states — and other symptomatology may similarly lead to contextual changes in impulsive choice computations that could precipitate a return to drug use. However, inconsistent findings on the relation between self-reported symptom severity and relapse to drug use suggest that measuring psychophysiological manifestations may add precision to the evaluation of current clinical states. We addressed these questions in a group of opioid users receiving standard treatment in an outpatient urban clinic. We explored whether different levels in reported craving, withdrawal symptoms, and anxiety, as well as heart rate variability, skin conductance, and facial electromyography were related to changes in choice behavior in a delay discounting task.

**Methods:** 22 Individuals with Opioid Use Disorder (OUD) who endorsed recent craving for heroin or other opioids were recruited to participate in 2 sessions. One was conducted before the participant received their medication and the other was conducted after. The order of the two sessions was randomized across subjects. On each session, we employed validated instruments to assess craving, subjective withdrawal symptom severity, and current levels of anxiety. Participants then completed a 12-minute delay discounting task. Heart rate variability (HRV), galvanic skin response (GSR), and corrugator and zygomatic surface electromyography (EMG) were measured during the task.

**Results:** We find that there were on average no significant differences in symptomatology or impulsive choice between the 2 sessions. However, using generalized linear mixed effects modeling, we find that discount rates in both sessions were significantly explained by anxiety levels ($p < 0.001$) and the number of craving episodes in the last 24 h ($p < 0.001$) and were inversely proportional to the length of those episodes ($p < 0.05$). Interestingly, HRV during the task was negatively correlated to craving intensity reported right before it (Spearman's rho = -0.37).

**Conclusions:** Taken together, these preliminary results point to a strong relationship between craving and discounting and suggest craving may have a physiologically measurable influence on impulsive decision-making. Current efforts in this study focus on increasing our sample size and establishing whether this and other psychophysiological measures are related to subjective value computations during the task.

**Keywords:** Opioid Addiction, Delay Discounting, Heart Rate Variability, Craving, Psychophysiology

**Disclosure:** Nothing to disclose.

### T242. Identifying Imaging Biomarkers of Resilience to Drug Use: Interaction Between Childhood Trauma History and Smoking Status on Gray Matter Structure in Adulthood

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**Background:** Cigarette smoking is the #1 cause of preventable death and disease in the United States and treatment outcomes for smoking cessation remain poor. The best public health tool to reduce smoking-related disease is primary prevention, including reducing and modifying risk factors. Childhood trauma (CT), such as maltreatment or abuse, is one of the strongest predictors of substance use disorders (SUDs) in adulthood, including cigarette smoking. However, not all individuals who experience CT develop SUDs or other psychopathologies. These so-called "resilient" individuals experience more adaptive long-term outcomes despite a history of trauma. Delineating the mechanisms of risk for, and resilience to, SUDs may inform the design and implementation of prevention strategies for at-risk youth. CT history and substance dependence are associated with overlapping structural and functional brain changes, suggesting that developmental trauma mechanistically alters the brain in a way that increases vulnerability to SUDs in adulthood. Here we considered structural variability in gray matter (GM) associated with relative resilience to cigarette smoking by comparing nonsmokers with a history of high levels of trauma to smokers with similar histories.

**Methods:** Structural brain images (T1-weighted MRI) and Childhood Trauma Questionnaire (CTQ) scores were collected at the NIDA-IRP (Baltimore, MD: 2003 - 2017) from 147 smokers and 149 nonsmokers who were matched for age, race, sex (Smk: 47% F; Nsmk: 42% F), and IQ. Scans were processed in Freesurfer using a standard processing pipeline and data quality control procedures, as described in the ENIGMA consortium, to produce cortical thickness, surface area, and subcortical volumes measures. A-priori region-of-interest (ROI) analysis was conducted in R for regions identified as sensitive to either smoking and/or CT (medial orbitofrontal; rostral middle frontal; caudal/rostral anterior cingulate; pars opercularis, pars triangularis, pars orbitalis; insula; frontal pole (FP); amygdala; hippocampus; thalamus, caudate, and nucleus accumbens (NAcc)). Linear models considered main effects and interactions of smoking status (nonsmoker vs. smoker) and CTQ subscale score quartiles (1st vs. 4th quartile) for Emotional Abuse (EA), Emotional Neglect (EN), Physical Abuse (PA) and Physical Neglect (PN). Within-group analysis for smokers only also modeled cumulative cigarette exposure and dependence severity. Model error terms included sex, age, IQ, and intracranial volume and all results were Bonferroni corrected for the number of ROIs tested ($N = 14$).

**Results:** There was a main effect of smoking status: smokers demonstrated greater NAcc volume compared to nonsmokers ($p = 0.007$). While controlling for age, there were within-smoker main effects of smoking severity: GM was directly correlated with age of smoking initiation in the ventrolateral PFC (pars triangularis; $p = 0.02$; pars orbitalis, $p = 0.04$), and caudate ($p = 0.02$); increasing pack years (cigarettes per day * number of years smoking) was associated with reduced GM in the insula ($p = 0.02$). With respect to CT, high EA scores were associated with larger caudate volume ($p = 0.04$). Finally, there was a PA score x smoking status interaction in the FP ($p = 0.04$); high PA scores were associated with reduced GM in nonsmokers, but increased GM in smokers.

**Conclusions:** We identified a presumptive neurobiological marker for resilience to nicotine drug use, despite high developmental risk as quantified by CT history. Specifically, there was a significant CT x smoking status interaction in the FP, a region involved in exploratory decision making and flexible/adaptive thinking in response to changing environment. FP cortical thickness normally decreases into late adolescence. Thus, increased cortical thickness in the CT-smoking group may reflect abnormal FP development, perhaps reduced synaptic pruning; whereas reduced thickness in the CT-nonsmoking group is consistent with a normal developmental trajectory, suggesting that normal FP development may serve as a protective factor promoting resilience to smoking or other SUDs. The finding is notable in the context of the latent vulnerability theory, which postulates that CT induces neurobiological adaptations that are beneficial in the context of abusive environments, but harmful in normative environments, increasing risk for psychopathology in adulthood. It is salient that structural development in the FP - a region involved in cognitive flexibility - is normalized in the relatively resilient nonsmoking group. Primary prevention strategies that focus on improving cognitive functions associated with the FP may reduce drug use in at-risk children.

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**Keywords:** Childhood Trauma, Nicotine Dependence, Gray Matter Volumes, Cortical Thickness, Structural MRI

**Disclosure:** Nothing to disclose.

### T243. Naloxone-Induced Precipitated Withdrawal is Associated With Increased Striatal Dopamine Release in Opiate-Dependent Men

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**Background:** Naloxone is an effective intervention for preventing overdose fatalities associated with opioid overdoses. Opiates target mu opioid receptors (MOR) which are prevalent in respiratory control regions in the pre-Bötzinger Complex of the brainstem (Smith et al., 1991; Pattinson, 2008). Naloxone antagonizes the effect of opioid agonists and restores breathing following opioid overdoses. Chronic history of opioid exposure results in upregulation of MOR inhibitory mechanisms which could be related to precipitated withdrawal experienced following acute naloxone injection. Excessive opiate use has been also associated with decreased DA receptor availability, but the role of dopamine in naloxone-induced withdrawal has been poorly investigated. Preclinical studies have reported dopamine increases (Iwamoto, et al., 1973), decreases (Spanagel et al., 1994), or no changes (Silverstone et al., 1993) during naloxone-precipitated withdrawal. In a previous PET study in opiate-dependent participants, we reported a non-significant trend of increases in dopamine release during naloxone-induced precipitated withdrawal, but the study was limited to using large regions of interest (ROIs) to measure changes in [11 C]raclopride specific binding (Wang, et al., 1997). Here we revisited the data previously published in Wang et al. (1997) to study voxel-level effects of naloxone on D2 receptor (D2R) availability during naloxone precipitated withdrawal.

**Methods:** Participants ($n = 10$; male $= 10$; age $= 41 \pm 4$ years) were included in the analysis who had at least one-year history of continues opiate use (heroin or methadone), met DSM-IV criteria for opiates dependence, and provided written informed consent. Participants underwent two consecutive PET imaging sessions (approximately 2 h apart) after injection of (up to) 8 mCi of [11 C] raclopride with a sequential dynamic scan (60 min). The baseline scan (first session) was performed following administration of

placebo (3 ml saline IV). The second session followed naloxone administration in increments of 0.1 mg/kg IV (every 4 min) until withdrawal symptoms appeared. Measures of cardiovascular function (e.g., blood pressure and pulse rate) and subjective measures of drug effects (e.g., desire to use opiates or loss of control) were recorded. PET images were processed in SPM for spatial alignment between sessions, motion correction, and normalization to the MNI space. In contrast to the previous a-priori region of interest (ROI) analysis (Wang et al., 1997), we identified ROIs by studying changes in standardized uptake value ratio (SUVr) with a cerebellar reference. Binding potential (BPnd) was calculated for the time activity of the identified ROIs by using simplified reference tissue model (Lammertsma and Hume, 1996) with a cerebellar reference.

**Results:** Naloxone administration induced precipitated withdrawal in participants, with significant changes in behavioral measures and cardiovascular function. Analysis of SUVr maps revealed two clusters bilaterally located within the caudate and putamen (pFWE < 0.01, corrected for cluster-size), which showed significantly less SUVr following naloxone administration. Analysis of BPnd for each of these clusters confirmed significant decreases in D2R availability during naloxone-induced precipitated withdrawal in these regions ($p = < 0.001$). Decreases in BPnd were associated with increases in "loss of control" and increases in systolic and diastolic blood pressure ($p < 0.05$, two-tailed).

**Conclusions:** Here we document significant decreases in D2R availability following naloxone-induced precipitated withdrawal which are consistent with increases in dopamine release. Our results are consistent with reports of such increases in brain DA in mice (Iwamoto, et al., 1973) and provide evidence that changes in D2R might contribute to certain physiological and psychological symptoms of withdrawal. Our findings may have implications for understanding the interactions between brain's opioid and DA systems and are relevant for the clinical management of naloxone-induced withdrawal symptoms.

**Keywords:** Raclopride, Buprenorphine-Naloxone, Dopamine D2 Receptors, Caudate, Opiate Addiction

**Disclosure:** Nothing to disclose.

### T244. Adverse Childhood Experiences Predict High-Intensity Binge Drinking Behavior in Adulthood

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**Background:** Recent work demonstrated high-intensity binge drinking – drinking beyond gender-specific binge thresholds –is rapidly increasing in the United States. While Childhood Trauma Experiences (CTEs) have been associated with alcohol-related behavior problems and comorbid psychiatric disorders, the relationship between CTEs and high-intensity binge drinking has not been examined. This study aims to analyze the relationship between CTEs and the risk of high-intensity binge drinking in adulthood using both clinical and population-based samples.

**Methods:** Subjects from the National Institute on Alcohol Abuse and Alcoholism (NIAAA) clinical sample ($n = 1,303$) and the National Epidemiological Survey of Alcohol and Related Condition–III (NESARC-III) ($n = 36,309$) were classified according to self-reported alcohol intake into four gender-specific, high-intensity binge groups based upon recent maximum alcohol consumption in a single day: non-binge, and levels I, II, or III. For the NIAAA sample, CTEs were assessed using the Childhood Trauma Questionnaire (CTQ) and Early Life Stress Questionnaire (ELSQ); for the NESARC-III, CTEs were assessed using the CTQ and Conflict Tactics Scale. Each sample was analyzed separately. Weighted prevalence rates of CTEs and comorbid psychiatric disorders among high-intensity binge groups were calculated for the NESARC-III. Multivariate multinomial logistic regression models, controlling for sociodemographic characteristics and comorbid psychiatric disorders, were used to examine the association between CTEs and high-intensity binge drinking for both NIAAA and NESARC-III samples.

**Results:** Analysis of the NIAAA sample showed subjects with maltreatment CTE were one to two times more frequent in binge level II, and two to five times more frequent in binge level III, compared with non-binge. These findings were confirmed in the NESARC-III sample, which showed maltreatment CTEs were 1.3 to 1.5 times more prevalent in level III and reported dysfunctional household CTEs 1.6 to 2.4 times more often, compared with non-binge. Comorbid psychiatric analyses of NESARC–III also showed that from non-binge to level III the prevalence of any substance use disorder increased from 18% to 85%; any mood disorder 12.2% to 22.2%; any anxiety disorder 12.5% to 16.4%, post-traumatic stress disorder increased from 4.1 to 8.6%; and any personality disorder, 11.9 to 33.8%.

Further NIAAA sample analysis showed statistically significant odds ratios (ORs) between CTEs and binge drinking level II and III that exceed 2 ($p < 0.05$ in level II and $p < 0.0001$ in level III) even after controlling for socio-demographics, while physical abuse also significantly predicted binge drinking level I ($p < 0.02$). Similarly, the NESARC-III sample showed statistically significant ORs for all maltreatment and all but one dysfunctional household CTE with binge level III (ORs = 1.3 ~ 1.9, with p-values < 0.0001) and binge level II (ORs = 1.2 ~ 1.4 with p-values = 0.03 ~ 0.0001).

**Conclusions:** CTEs significantly increased the risk for high-intensity binge drinking both in a clinical sample and a large population-based sample, suggesting individuals with CTEs are at increased risk for health consequences related to extreme alcohol consumption. Further investigations into the relationship and underlying mechanisms between CTEs, high-intensity binge drinking, and comorbid psychiatric disorders is needed to improve our understanding of the biology and prevention/treatment of this emerging harmful alcohol use pattern.

**Keywords:** Childhood Trauma, Binge Drinking, Comorbidity

**Disclosure:** Nothing to disclose.

### T245. Neurodevelopmental and Synaptic Modifications in Tropomodulin-2 Knockout Mice Results in Addiction-Relevant Phenotypes

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**Background:** Addiction or substance abuse disorder is characterized by maladaptive behavioral fixation and loss of control to withhold drug usage despite negative consequence. Alterations in synaptic and intrinsic membrane properties of neurons in brain's reward-circuit are considered to be the cellular basis of addiction. These structural and functional changes in neurons are, in part, regulated by actin-cytoskeleton machinery. Therefore, a preexisting or drug-induced deficit in actin-regulation could increase the vulnerability to addiction. We analyzed behavior and physiology data from the Knockout Mouse Project (KOMP) at The Jackson Laboratory to discover a cluster of genes that have behavioral deficits that are predictive of addiction phenotypes. We carried out detailed addiction relevant phenotyping of several candidates

and present results of Tropomodulin 2 (TMOD2) knockout. TMOD2 has previously been implicated in human studies as a regulator of addiction phenotypes. It is highly expressed, actin-regulating gene of nervous system that specifically caps and stabilizes F-actin pointed ends to prevent its elongation and depolymerization. It plays an important role in shaping dendritic complexity and dendritic spine morphology.

**Methods:** We carried out a battery of behavioral test, electrophysiology in adults and embryonic neuronal cultures, adult brain MRI, and biochemistry to interrogate the mechanism through which TMOD2 regulates cocaine responses.

**Results:** TMOD2 KO mice exhibit novelty-induced hyperactivity or sensation-seeking phenotype in open field assay along with anxiolytic traits demonstrated by increased center-exploration. Cocaine-induced locomotor sensitization was further attenuated in TMOD2 KO compared to C57BL/6NJ WT. TMOD2 KO mice performed poorly in cocaine IVSA test but in two-bottle sucrose preference test demonstrated their intact preference towards natural reward. RNAseq analysis in cortical and striatal punches revealed an upregulation in developmental gene En2 and downregulation of inhibitory synapse-forming gene Npas4 in TMOD2 KO mice. Developmentally regulated body-weight and whole-brain volume was found to be significantly reduced in TMOD2 KO. Strikingly, the white matter proportion in brain was significantly reduced in TMOD2 KO representing axonal/myelin scarcity. Spontaneous spiking activity in P0-P1 cortical culture was found to be significantly more in TMOD2 KOs, recapitulating the developmental deficits. mEPSC amplitude was elevated in drug-naïve TMOD2 KO, whereas anticipated drug-induced increase in mEPSC frequency was lacking in caudomedial accumbens shell. Additionally, in cocaine-naive state, accumbens neurons exhibit higher mIPSC frequency, which following cocaine exposure was further amplified in TMOD2 KO. This amplified inhibitory tone in TMOD2 KOs in basal and following cocaine exposure could resist the increase in cocaine-induced excitatory tone on accumbens neurons leading to attenuated sensitization response.

**Conclusions:** TMOD2 deletion resulted in structural and synaptic dysregulation along with several addiction-relevant phenotype. Actin-regulating genes specifically expressed in brain appears to be strong candidate for understanding neurobehavioral abnormalities associated with addiction.

**Keywords:** Cocaine Addiction, Mouse Models, Actin Remodeling, MRI, Neurodevelopment

**Disclosure:** Nothing to disclose.

### T246. Higher Plasma Cholesterol Levels in Alcohol Use Disorder are Associated With TSPO Polymorphism and With Withdrawal Severity

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**Background:** The outer mitochondrial membrane 18 kDa translocator protein (TSPO) is involved in steroid synthesis and provides a binding site for cytoplasmic cholesterol. The functional TSPO polymorphism rs6971 has been associated with reduced cholesterol transport into the mitochondria. Furthermore, TSPO rs6971 affects the affinity of various TSPO radiotracer binding (e.g. [11 C] PBR28) and has shown involvement in diurnal variation in cortisol in AUD. Recently, we found a negative correlation between [11 C] PBR28 PET and plasma cholesterol in a small sample of patients with alcohol use disorder (AUD), an effect dependent on rs6971.

**Methods:** Here, we aim to investigate whether rs6971 is associated with cholesterol regulation in a larger sample of n = 926 participants with an AUD (current DSM IV or 5 diagnosis as per SCID) and n = 553 non-dependent participants.

**Results:** We found that, first, AUD participants showed higher levels of total cholesterol, HDL, and triglycerides than non-dependent participants (all $p < 0.01$; corrected for age and BMI), but there were no group differences in LDL cholesterol. Second, for the AUD group only, there was an effect of rs6971 on total cholesterol, LDL, and triglycerides (all $p < 0.05$; corrected for age and BMI); but not on HDL; with higher levels for AA (n = 61) > AG (n = 320) > GG (n = 547). Moreover, within the AUD group, withdrawal scores measured with CIWA were higher in AA > AG > GG ($p < 0.05$); and HDL ($r = 0.26$, $p < 0.0001$), LDL ($r = -0.12$, $p = 0.002$) and total cholesterol at trend level ($r = 0.07$, $p = 0.08$) were correlated with CIWA scores.

**Conclusions:** These findings are in line with previous studies on elevated plasma cholesterol levels in AUD and reveal for the first time an association between TSPO rs6971 genotype and cholesterol levels and show evidence of an association between TSPO and cholesterol levels with withdrawal severity in human AUD.

**Keywords:** TSPO, Alcohol, Cholesterol Biosynthesis

**Disclosure:** Nothing to disclose.

### T247. Changes in Prefrontal Glutamate, Glutamine, and GABA Levels From 72 h to 7 Days Post-Abstinence in Treatment NAÏVE Alcohol Dependent Individuals

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**Background:** Studies of advanced alcohol dependence (AD) have demonstrated disturbances in glutamatergic neurotransmission that appear to be highly dynamic during early abstinence from alcohol. However, most of these studies have been cross-sectional and limited to individuals with severe AD. Investigations of GABA and glutamine levels in AD have been rare because in vivo measurement of these metabolites is technically challenging. We previously presented data demonstrating a statistically significant, 9% increase in prefrontal GABA levels in treatment-naïve AD individuals who completed proton-MRS scans: 1) 12-24 h and 2) 60-72 h following their last drink. The present study reports data from a 3rd and final proton-MRS scan from this participant cohort that occurred approximately 7 days following participants' last drink.

**Methods:** Twenty-three treatment-naïve individuals (18 male, mean age [sd] = 27.0 [6.0]), who met criteria for DSM-IV AD and reported ≥ 25 drinks/week, completed > 1 scan; 21 of these individuals completed all three scans. Exclusion criteria included current DSM-IV Axis I disorder and positive urine-drug or alcohol-breath screens. For scan 1, participants had to provide a urine ethyl-glucuronide (EtG) level > 200 ng/ml, corroborating self-reported drinking within 24-hours. For scans 2 and 3, participants had to provide a urine EtG level < 200 ng/ml, corroborating self-reported abstinence since their previous scan. Each scan included two-dimensional j-resolved proton-MRS acquisitions (Siemens-3T) in a 25 × 25 × 30 mm dorsal anterior cingulate cortex (dACC) voxel. Neurometabolite concentrations were estimated using the ProFit algorithm, scaled to water, and corrected for cerebrospinal fluid content. Craving was measured with the Alcohol Urge Questionnaire.

**Results:** 1) There were no statistically significant differences between participants scan 2 and scan 3 glutamate (1.8% mean increase, $t = 0.58$, $p = 0.566$), GABA (0.3% mean increase, $t = 0.07$, $p = 0.948$) or glutamine (2.4% mean increase, $t = 0.559$, $p = 0.582$) levels. 2) Participants with lower levels of GABA, glutamine, and glutamate at scan 2 experienced significantly greater increases in GABA ($r = -0.70$, $p < 0.001$), glutamine ($r = -0.78$, $p < 0.001$), and glutamate ($r = -0.77$, $p < 0.001$), respectively, between scans 2 and 3. 3) Changes in prefrontal glutamine levels between scan 1 and scan 2 were prospectively associated with changes in self-reported alcohol craving between scan 2 and scan 3, such that individuals with larger increases in glutamine tended to subsequently report larger decreases in alcohol craving ($r = -0.46$, $p = 0.038$).

**Conclusions:** Treatment-naïve individuals with AD experienced no statistically significant changes in prefrontal GABA, glutamate, or glutamine levels from approximately 72 h to 7 days post-abstinence from alcohol. We recently presented data from the same participant cohort demonstrating statistically significant increases in prefrontal GABA levels from approximately 12 to 72 h post-abstinence, as well as significantly decreased levels of prefrontal GABA in AD participants at 12 h post-abstinence relative to light drinkers. Together, these findings suggest that treatment-naïve AD individuals may experience a durable normalization of prefrontal GABA levels within 12 h of their last drink. Results from the present study also support a prospective association between normalization of prefrontal glutamine levels following abstinence from alcohol and decreased self-reported alcohol craving. If replicated in larger samples, these findings could have implications for pharmacotherapy of drugs working on these systems.

**Keywords:** Alcohol Use Disorder, Proton Magnetic Resonance Spectroscopy, Abstinence, Craving, Treatment-Naive

**Disclosure:** Nothing to disclose.

### T248. Opioid Antagonist Treatment Modulates Brain Response to Monetary Reward

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**Background:** Opioid addiction affects up to 5 million individuals in the US alone. Addiction is associated with sensitization to drug-related stimuli and abnormal brain processing of reward. Nucleus Accumbens (NAcc) is a key brain structure in the brain system mediating the response to rewarding stimuli, both natural and drug-related (1, 2). Opioid antagonists are effective for relapse prevention in opioid addiction. However, it is not entirely clear whether they may also attenuate normal hedonic responses (3) and thus patients' normal motivation. We examined the impact of treatment with an injectable extended release opioid antagonist naltrexone (XR-NTX) on brain responses to monetary rewards in detoxified patients with opioid use disorder (OUD) who have been addicted to heroin.

**Methods:** Twenty-one patients with OUD underwent two functional magnetic resonance imaging (fMRI) sessions while performing the "monetary card-guessing" task" (MCGT) that consistently activates NAcc (4). The first fMRI session was completed prior to XR-NTX treatment and the second was approximately two weeks after XR-NTX treatment was started. In each MCGT trial, participants viewed the back of a card and guessed whether the front of the card would be revealed be of red or black suit. Correct guesses gained $10.00, and incorrect guesses lost $9.75. A slightly greater amount was used for wins than losses to avoid potential task frustration while keeping 50/50 outcome probabilities. Each individual trial contained 2 parts, a guessing phase (2 s) and an outcome phase (2 s) that were separated by a variable inter-trial delay (range 2-12 s, mean 5 s). Responses and reaction times were recorded with a 2-button response pad. Based on apriori hypotheses regarding NAcc, we conducted a NAcc region of interest analysis. NAcc was defined anatomically using the Harvard Oxford subcortical atlas. Mean scaled beta coefficients (% BOLD signal change) from each significant cluster in the respective Win and Loss maps were extracted for graphic examination and further statistical testing. Participants' craving for opioids was recorded in each session.

**Results:** We found that opioid antagonist treatment did not change the NAcc brain responses to either winning or losing in (left, $p = 0.272$; right, $p = 0.365$) in the OUD patients. Winning elicited greater brain responses than losing (left, $p = 0.000018$; right, $p = 0.000006$). There was no treatment by condition interactions (left NAcc, $p = 0.129$; right NAcc, $p = 0.491$). Craving for heroin was reduced ($p = 0.000024$) on XR-NTX treatment. There were no correlations between brain responses to winning or losing and the self-reported heroin craving.

**Conclusions:** Patients with opioid use disorder showed greater NAcc activation to wins than losses, which may be contrary to the decision-making pattern dominated by loss aversion reported in healthy individuals (5), an observation that could be confirmed in a controlled study. Opioid antagonist treatment reduced subjective heroin craving, while it didn't affect the brain responses to winning and losing, suggesting that XR-NTX does not impair normal reward processing.

**Keywords:** Opioid Addiction, Extended-Release Naltrexone, Monetary Reward, Nucleus Accumbens, Functional MRI (fMRI)

**Disclosure:** Alkermes PLC, Advisory Board

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### T249. Genetic and Neuroanatomical Basis of Comorbidity Between Depressive, Substance Use and Chronic Pain Disorders

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**Background:** Clinically, psychiatric co-morbidity impacts many aspects of patient care, a problem which is only compounded when considering the relationships between psychiatric comorbidity, pain disorders and other medical conditions. There is

evidence that similarities in genetic risk factors account for a significant portion of co-morbidity in general, but there have been few systematic studies that compare this genetic basis across co-morbid conditions in a community sample. Specific genetic loci that mediate co-morbidity are generally unknown. Finally, it is unknown the extent to which co-morbidity is reflected in disease processes affecting similar neuroanatomical correlates, e.g. similar patterns of alterations in cortical thickness compared to unaffected individuals.

**Methods:** This study was based on the Genetics of Brain Structure and Function Study (GOBS) sample, comprised of extended pedigrees of Mexican American descent living in San Antonio, TX. Approximately 1,500 individuals completed the Mini-International Neuropsychiatric Interview (MINI), provided information about past medical history, and underwent both high resolution structural MRI as well as genotyping of genome-wide single nucleotide polymorphisms (SNPs). FreeSurfer software was used to generate maps of thickness across the surface for each individual. SOLAR software was used to estimate pairwise genetic correlations between each of 35 chronic medical and psychiatric illnesses (595 total pairs). The genetic correlations between cortical thickness and clusters of co-morbid conditions were also calculated.

**Results:** As expected, there was a rich set of phenotypic correlations (comorbidity) between conditions. Highly significant correlations included those between medical conditions with known etiological overlap (for example, hypertension, hyperlipidemia, and diabetes) as well as within substance use disorders and affective disorders, respectively. Chronic pain was strongly comorbid with medical, affective and substance use disorders. Of the ~50 significant phenotypic correlations between diseases that are highly heritable in the univariate sense, only 10 revealed significant genetic correlations after Bonferroni correction for multiple comparisons, including associations between: nicotine use disorder and chronic pain disorder; major depressive disorder and past suicide attempt (which was treated as a distinct clinical phenotype); alcohol use, cocaine use, and marijuana use disorders. Linkage analysis based on SNP array data did not detect specific genetic loci mediating the genetic correlation between pain and nicotine use disorders at the threshold for genome-wide significance.

**Conclusions:** Co-morbidity between chronic pain, substance and mood disorders has a strong basis in shared genetic factors. Neuroinflammatory risk factors may mediate comorbidity between pain disorders, affective disorders and substance use disorders; however, the current sample size may be insufficient to adequately test this hypothesis. The next phase of this study will compare neuroimaging phenotypes of co-morbid conditions, to determine whether specific neuroanatomical phenotypes are related to genetic risk across conditions.

**Keywords:** Chronic Pain, Imaging Genetics, Substance Abuse Disorders, Major Depressive Disorder (MDD)

**Disclosure:** Nothing to disclose.

### T250. Persistence Doesn't Always Pay: Sustained Brain Response to 500 ms Drug Cues Predicts Future Drug Use

*Paul Regier*, Kanchana Jagannathan, Stefanie Darnley, Elliott Berkowitz Sturgis, Matthew Taylor, Jesse Suh, Charles O'Brien, Anna Rose Childress*

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**Background:** Overdoses associated with cocaine use are on the rise, many of which are due to cross-contamination or purposeful mixing of fentanyl with cocaine. This deadly new trend makes relapse an increasingly risky event for individuals with cocaine-use disorders. Understanding the brain response to drug cues associated with future drug use should expedite effective treatment development to help prevent relapse. We hypothesized that an elevated response to drug cues in relapse-relevant regions would predict future cocaine use.

**Methods:** This analysis included 73 treatment-seeking cocaine patients (primarily African-American males). These patients were scanned with fMRI during an inpatient period and then monitored for drug use during an 8-week outpatient period following hospital discharge. Brain response to brief 500 ms cocaine (and other evocative) cues (as compared to neutral cues) were examined for their relationship to drug use outcome. In addition to utilizing data from the whole fMRI time series of the 500 ms task, the data were also divided into two halves: the first half contained 24 of each evocative (drug, sexual, aversive) and neutral cue type; the second half contained these same 24 of each cue type, although repeated in a pseudorandom order. This approach using first and second halves allowed us to examine whether the brain response to drug cues would reduce with repeated presentation (the most common response to repeated stimuli), or whether the brain response to drug might persist or even increase across the task – a potential relapse-relevant pathology.

**Results:** Consistent with our hypothesis, a greater brain response to 500 ms drug (vs. neutral) cues in the whole time series in several relapse-relevant regions (e.g., amygdala (r:), striatum (r:), hippocampus (r:), midbrain (r:) predicted more drug use in the 8-week outpatient phase. Interestingly, analysis of the two halves of the task revealed that the heightened brain response to cocaine cues associated with future cocaine use was the most robust in the second half of the task.

**Conclusions:** The brain response to brief 500 ms drug cues can predict future relapse. The dramatic effect in the second half of the task is driven by a sustained or increasing response (or both) by those with worse drug outcomes. On the other hand, those with better drug-use outcomes have a decreasing pattern of brain response across the task. This sustained or increasing response to repeated cues may indicate an underlying pathology linked to relapse vulnerability. Targeting this unique response may help to prevent future drug use and to reduce the incidence of fatal drug overdose.

**Keywords:** Cocaine, Functional MRI (fMRI), Drug Cues

**Disclosure:** Nothing to disclose.

### T251. Analysis of Subcortical Volume and Morphology in Varying Levels of Gambling Behavior

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**Background:** The clinical phenotype of gambling disorder (GD) is suggestive of changes in brain regions involved in reward and impulse suppression, notably the striatum. Studies have yet to characterize striatal morphology (shape) in GD and whether this may be a vulnerability marker.

Aims: To characterize morphology of the striatum in those with disordered gambling (At-Risk Gambling and GD) versus controls.

**Methods:** Individuals aged 18–29 years were classified a priori into those with some degree of gambling disorder symptoms (At-Risk Gambling and Gambling Disorder), or controls. Exclusion criteria were current mental disorder (apart from GD), history of brain injury, or taking psychoactive medication within 6 weeks of enrollment. History of any substance use disorder was exclusionary. Participants completed an impulsivity questionnaire and

structural brain scan. Group differences in volumes and morphology were characterized in subcortical regions of interest, focusing on the striatum.

**Results:** 32 people with gambling disorder symptoms (14 At-Risk and 18 Gambling Disorder participants) and 22 controls completed the study. Gambling Disorder symptoms were significantly associated with higher impulsivity; and with morphological alterations in bilateral pallidum and left putamen. Localized contraction in the right pallidum strongly correlated with trait impulsivity in those with gambling disorder symptoms.

**Conclusions:** Morphologic abnormalities of the striatum appear to exist early in the disease trajectory from subsyndromal gambling through to GD, and thus may constitute candidate biological vulnerability markers, which may reflect differences in brain development associated with trait impulsivity. Striatal morphology and associated impulsivity might predispose to a range of problematic repetitive behaviors including gambling.

**Keywords:** Morphology, Human Neuroimaging, Gambling Disorder

**Disclosure:** Takeda, Grant, American Psychiatric Publishing, Royalties, Johns Hopkins Press, Royalties, Oxford University Press, Royalties, Biohaven, Grant

### T252. Brain Response to Baby Schema in Methadone-Maintained Mothers

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**Background:** Opioid abuse and dependence (OD) have reached epidemic proportions in US. OD involves impaired interpersonal interactions and social cognition. In particularly, mothers with OD exhibit deficits in care giving such as reduced pre- and post-natal engagement with the baby and physical and emotional child neglect, leading to high utilization of foster care. Though such deficits have intergenerational effects on children, families and society at large, our knowledge on the phenomenology and neurobiology of maternal behaviors in OD mothers is limited. Methadone, an opioid agonist, is the treatment of choice for most OD mothers. However, its impact on caregiving motivation of OD mothers is unknown.

**Methods:** We compared brain responses to baby schema in OD mothers and socio-demographically matched drug-free controls, utilizing functional magnetic resonance imaging (fMRI). Baby schema was operationalized as high (i.e. round face, high forehead, big eyes, small nose and mouth) and low (i.e. narrow face, low forehead, small eyes, big nose and mouth) baby schema portraits. Participants' care giving motivation was assessed by a question "How much do you want to take care of this baby?" using a 7 points likert scale.

**Results:** We studied 16 OD mothers ($30.00 + 3.82$ y/o, 75% Caucasian, 6% African American, 19% mixed races, $11.64 + 0.95$ years of education) and 16 healthy females ($31.82 + 7.80$ y/o, 44% Caucasian, 44% African American, 13% Asian and 6% mixed races, $14.00 + 3.32$ years of education). Repeated measures ANOVA found no Group by Baby Schema interaction ($p = 0.66$). OD mothers reported same level of caregiving motivation towards faces with high and low baby schema ($p = 0.06$) while healthy mothers reported slightly higher caregiving motivation towards the high baby schema faces ($p = 0.04$). Compared to healthy counterparts, OD mothers showed reduced responses in the ventral striatum, a brain area previously linked to baby schema response in healthy controls. In addition, OD mothers showed decreased brain activations in the precuneus, anterior cingulate, middle and medial frontal cortex ($z = 1.64$, uncorrected).

**Conclusions:** Our preliminary findings suggest that opioid agonist treatment modulates brain responses to baby schema in OD mothers.

**Keywords:** Opioid Agonist Treatment, Functional MRI (fMRI), Baby Schema, Care Giving

**Disclosure:** Nothing to disclose.

### T253. Hippocampal Subfield CA2 + 3 is Sensitive to Age-By-Alcoholism Interactions

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**Background:** Volumes of subregions relative to total hippocampal volume might can augment differentiation of disease processes. For example, damage to hippocampal subfield cornu ammonis 1 (CA1) is often reported in Alzheimer's disease (AD), whereas CA4/dentate gyrus is described in response to trauma and post-traumatic stress disorder. Two previous studies have explored the effects of chronic alcohol use on hippocampal subfields: one reported smaller volume of the CA2 + 3 in alcohol-dependent subjects relative to controls, associated with years of alcohol consumption; the other smaller volumes of presubiculum, subiculum, and fimbria in alcohol-dependent relative to control men.

**Methods:** The current study, conducted in 24 adults with DSM-5 diagnosed alcohol use disorder (AUD, 7 women, $53.7 \pm 8.8$) and 20 controls (7 women, $54.1 \pm 9.3$), is the first to use FreeSurfer 6.0, which provides state-of-the art hippocampal parcellation, to explore the sensitivity of hippocampal subregions to alcoholism. T1 weighted CSF-nulled MPRAGE and T2-weighted 3D Fast Spin Echo (T2 Cube) were collected on a GE MR750 system with a 32-channel Nova head coil. FreeSurfer 6.0 hippocampal subfield analysis produced 12 subfields: parasubiculum; presubiculum; subiculum; CA1; CA2 + 3; CA4; GC-ML-DG (Granule Cell (GC) and Molecular Layer (ML) of the Dentate Gyrus (DG)); molecular layer of hippocampus (ML); hippocampus-amygdala-transition-area (HATA); fimbria; hippocampal tail; hippocampal fissure; and whole volume for left and right hippocampi.

**Results:** Multiple regressions for raw volumetric data for each subregion by diagnosis (AUD vs. control), age, sex, hemisphere, and supratentorial volume (svol) showed significant effects of svol ($p < 0.04$) on nearly all structures (excluding tail and fissure). Svol-corrected volumes showed effects of age (GC-ML-DG, HATA, fimbria, fissure, $p < .03$), diagnosis (subiculum, CA1, CA4, GC-ML-DG, ML, HATA, fimbria, whole hippocampus, $p < .03$), and sex (fimbria, $p < .03$); CA2 + 3 showed a diagnosis by age interaction ($p = .03$) indicating older AUD had a smaller volume than would be expected for their age. Because neither svol nor hemisphere effects were significant and sex effects were minimal, hippocampal-subregion data were corrected for svol, hemisphere, and sex for further analyses. In these AUD participants, serum calcium (Ca2 + ) levels were lower than in the controls ($p = .02$). In the AUD group only, lower Ca2 + correlated with smaller volumes of CA1 ($\rho = .55$, $p = .007$), and nominally correlated with smaller volumes of CA2 + 3 ($\rho = .42$, $p = .05$) and CA4 ($\rho = .40$, $p = .06$).

**Conclusions:** The current results concur with the previous study, identifying CA2 + 3 as sensitive to alcoholism and its interaction with age and provide initial evidence for an imaging phenotype distinguishing AUD from AD and trauma.

**Keywords:** Alcohol Use Disorders, Hippocampus, Hippocampal Subfields, Alzheimer's Disease

**Disclosure:** Nothing to disclose.

### T254. Preliminary In-Vivo Evidence of Neuroimmune Activation in Chronic Pain States in Humans: Analgesic Reversal With Autologous Stem Cell Treatment

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**Background:** Each day approximately 115 Americans die from opioid overdose and 40% of these deaths involve opioids prescribed for treatment of pain states. Existing evidence suggests activation of brain microglia (and subsequent release of pro-nociceptive proteins (e.g. IL-1β, IL-18)) enhances both susceptibility to chronic pain states and tolerance to opioid analgesia, two culprits of the nationwide opioid epidemic. However, while compelling, much of this evidence derives from basic research in animal models with a paucity of such evidence in humans. In-vivo confirmation in humans is required in order to drive development of novel, more efficacious, evidence-based, personalized treatment strategies in debilitating chronic pain syndromes.

**Methods:** To determine whether brain immune processes are upregulated within specific brain regions in individuals experiencing clinically relevant pain symptoms, we completed 11C-PBR28 PET scanning in 10 female human volunteers with and without chronic pain. Participants were pre-screened for the TSPO gene variant (rs6971) known to impact PBR-28 binding. We hypothesized that, compared to pain-free volunteers, those experiencing pain have enhanced neuroimmune activity within specific brain regions previously identified for their critical role in regulating the human pain experience (thalamus, amygdala, hippocampus, nucleus accumbens). Further, we predicted that enhanced neuroimmune activity within these brain regions should be more closely related to their pain symptoms than to their specific diagnosis. Quantification of 11C-PBR28 PET data involved application of standardized uptake volume ratio (SUVR) technique, accounting for binding affinity. Only moderate and high affinity binders (as determined by rs6971 screening) completed 11C-PBR28 PET scanning. Subsequent data processing involved standardized co-registration of participant's 11C-PBR28 SUVR data with their MRI data and subsequent normalization to a standardized template (MNI). 11C-PBR28 was compared between groups (pain vs. no pain) on a whole brain, voxel-by-voxel basis within MATLAB using SPM (Statistical Parametric Mapping) tools. Each participant's pain experience (or lack thereof) was quantified via completion of the McGill Pain Questionnaire (MPQ) prior to 11C-PBR28 PET neuroimaging. Of the 10 participants, 4 had a diagnosis of Major Depression (MDD), 2 had a diagnosis of Post Traumatic Stress Disorder, 2 had a history of Traumatic Brain Injury (TBI) (automobile accident 1 year prior to scanning), 1 had history of ischemic stroke (6 months prior to scanning), and 2 participants were without a history of neuropsychiatric illness (Healthy Controls). Of the 10 participants, 3 participants ($n = 1$ MDD, $n = 1$ TBI, and $n = 1$ stroke) reported clinically relevant, chronic pain symptoms as quantified by the MPQ. Additionally, of the 2 TBI participants, 1 received autologous stem cell treatment following recovery and 1 received placebo (in place of stem cell treatment).

**Results:** Results from whole brain voxel by voxel ANOVA revealed regional differences (F-tests) in PBR-28 SUVR when comparing participants with pain against those without pain as follows: Left Thalamus (xyz:-6,-10,10; $k = 53$; $F = 112$; $p < 0.001$); Right Thalamus (xyz:16,-26,8; $k = 60$; $F = 48$; $p < 0.001$); Right Parahippocampal Gyrus (xyz: 20,-12,-16; $k = 109$; $F = 26$; $p = 0.001$); Dorsal Brain Stem (xyz: -2,-34,-32; $k = 28$; $F = 22$; $p = 0.001$). Extracted data shows higher PBR-28 SUVR in each of the above regions region in all participants with chronic pain (compared to all pain free participants). The TBI participant that received stem cell treatment experienced resolution of pain symptoms whereas chronic pain persisted unabated in the participant that received placebo (in lieu of stem cells). Additionally, the untreated TBI participant exhibited enhanced PBR-28 SUVR within the above brain regions compared to the TBI participant that received stem cell treatment.

**Conclusions:** In sum, the findings outlined above represent localized enhanced 11C-PBR28 SUVR data in individuals with chronic pain, localized to brain regions wherein neuroimmune activity is believed to play a critical role in processing peripheral nociceptive stimuli. That the findings are symptom specific (as opposed to related to particular diagnoses) further underscores a potential role of neuroimmune interactions in pain states across several diagnoses. Although this is a small, preliminary study, the results replicate substantial evidence from animal models outlining pain specific enhanced neuroimmune activity in brain regions component to pain regulatory circuitry (i.e. pontine reticular formation, para-hippocampal gyrus, bilateral thalamus). Additionally, the very preliminary evidence suggestive of analgesic action of autologous stem cell treatment and its relation to reversal of brain regional neuroimmune activation in TBI patients further underscores the immense clinical translational potential of these findings. Expansion of this study is ongoing.

**Keywords:** Microglial Activation, Chronic Pain, Inflammation, Biomarker, Adult Stem Cells, TSPO and [11 C]PBR-28 PET, TBI

**Disclosure:** Nothing to disclose.

### T255. Reduced Segregation Between Cognitive and Emotional Processes in Cannabis Dependence

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**Background:** Addiction is characterized by an erosion of cognitive control towards drug taking that is accentuated by negative emotional states. Here we tested the hypothesis that enhanced interference on cognitive control reflects a loss of segregation between cognition and emotion in addiction.

**Methods:** We analyzed neuropsychological and high-resolution imaging data from the Human Connectome Project in 1204 adults aged 22-35 (54.5% female; mean [SD] age, 28.8 [3.7] years), including 89 individuals with cannabis dependence, 89 matched controls and 87 recreational cannabis users. Composite behavioral and imaging measures of cognition and emotion were derived using principle component analysis. We examined the relationship between these measures, and then examined multivariate brain-behavior associations using canonical correlation analysis, testing whether cognition and emotion are linked in a sample with drug addiction.

**Results:** In the cannabis-dependent group, composite measures of cognition and emotion were significantly correlated, such that trait negative emotionality was associated with poor cognition ($r = 0.40$, $p < .001$), and brain activations to emotional stimuli were associated with activations to cognitive demand ($r = .29$, $p = .007$). These measures were uncorrelated in matched controls and in non-dependent recreational cannabis users. In the cannabis-dependent group there was a substantial overlap between cognitive and emotional brain-behavior associations ($r = .52$, $pFWE < .001$), but in the controls the associations were mostly restricted to the cognitive domain.

**Conclusions:** These findings support our hypothesis of impaired segregation between cognitive and emotional processes in cannabis dependence that might contribute to poor cognitive control under conditions of increased emotional demand. Interventions aimed at buffering negative emotionality and/or strengthening cognitive control might help restitute the loss of segregation between emotional and cognitive networks in addiction.

**Keywords:** Addiction, Cannabis Dependence, Functional MRI (fMRI), Cognition, Emotion

**Disclosure:** Nothing to disclose.

### T256. In Vivo Evidence of Aberrant Synaptic Plasticity in Cocaine Users Vs. Healthy Controls Using 11C-UCB-J PET

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**Background:** Seminal studies in rodents have shown robust and long lasting ( > 1 month) alterations in dendritic spines in various brain regions (e.g., increases in nucleus accumbens (NAc) and medial prefrontal (mPFC), decreases in orbitofrontal cortex (OFC) after experimenter- or self-administered stimulants/cocaine. Such drug-induced aberrant synaptic plasticity has been hypothesized to underlie the enduring and maladaptive behaviors (e.g., drug craving, compulsive use, recurrent relapse) observed in humans addicted to the drug. To date, however, in vivo studies of such microanatomical changes in clinical populations have been hindered by the lack of suitable, non-invasive neuroimaging tools. Our group has recently validated the use of 11C-UCB-J for imaging synaptic density in humans in vivo using positron emission tomography (PET). 11C-UCB-J binds to synaptic vesicular glycoprotein 2a (SV2a), a ubiquitous presynaptic vesicular membrane protein in neurons. Ex vivo studies in non-human primates have shown significant regional correlations between 11C-UCB-J binding/PET imaging outcome measures and binding of synaptophysin, a "gold standard" in vitro marker of synaptic density. The aim of the current study was to test, for the first time in living humans, whether synaptic density as measured by 11C-UCB-J PET is altered, either in previously hypothesized regions (e.g., ventral striatum (VS), mPFC and OFC) or others not previously examined, in subjects with cocaine use disorder (CUD) as compared to healthy controls (HC).

**Methods:** Subjects with DSM-5 CUD (N = 15) underwent a single 11C-UCB-J PET scan after two weeks of urine toxicology verified inpatient abstinence. Individually age-, sex-, and race-matched HC (N = 15) were scanned as outpatients. All subjects had arterial sampling and full radiometabolite analyses in order to derive measures of the specific-to-non-displaceable binding potential (BPND), using a white matter region (centrum semiovale) as a reference, and the total volume of distribution (VT) corrected for the free fraction of the ligand in plasma (VT/fP). PET scans were acquired for 120 min on the HRRT scanner after a single intravenous 20 mCi radiotracer bolus. Subjects had structural magnetic resonance images (MRI) acquired for coregistration with PET scans and region of interest (ROI) analyses using the automated anatomical labeling (AAL) template. Between-group statistical comparisons of primary outcome measures (BPND and VT/fP) in hypothesized regions were conducted using independent two-tailed, t-tests, as were more exploratory secondary analyses in other AAL regions. Hypothesis: Individuals with CUD would have elevated VT/fP and BPND in VS and mPFC and reduced measures in OFC as compared to matched HC.

**Results:** 15 CUD subjects (43 ± 7 years; 3 F/12 M; 9 African American, 5 Caucasian, 1 Hispanic) with 18 ± 9 years cocaine use of 18 ± 7 days/month were scanned after 15 ± 3 days of abstinence and compared to individually matched HC (43 ± 9 years; 3 F/12 M; 9 AA, 5 C, 1 H; p = NS). Statistical analyses revealed significant reductions in regional BPND (3.0 ± 0.3 vs. 3.5 ± 0.6; p = 0.02) and VT/fP values (70 ± 7.7 vs. 79 ± 10.7; p = 0.01) in medial OFC. No alterations were noted in mPFC or VS. Of note, VT measures in white matter (centrum semiovale) were identical in CUD and HC subjects (6.2 ± 1.3 vs. 6.2 ± 0.8). Exploratory analyses of additional AAL ROIs, however, demonstrated more widespread, statistically meaningful alterations in synaptic density, including reductions in calcarine fissure, cingulate cortex, operculum, fusiform gyrus, occipital cortex, olfactory cortex, rectus, and temporal lobe and elevations in locus coeruleus and raphe nuclei in CUD (p ≤ 0.10 in 29 and p ≤ 0.05 in 13 of 95 regions for BPND, and in 22 and 8 regions, respectively, for VT/fP).

**Conclusions:** This is the first imaging study in humans to examine the effects of chronic cocaine use on brain measures of synaptic density in vivo. Results are consistent with broad alterations in synaptic density in CUD that partially overlap with those identified in preclinical (rodent) studies. Exploratory analyses suggest intriguing differences in cortical (lower) vs. brainstem (higher) measures. Further analyses are warranted in order to control for the potentially confounding effects of grey matter differences (e.g., using partial volume correction) and multiple comparisons (e.g., using voxel-based methods). If confirmed, these findings would provide powerful clinical translational support for the drug-induced aberrant synaptic plasticity hypothesis of addiction.

**Keywords:** Cocaine-Related Disorders, Synaptic Density, PET Imaging Study

**Disclosure:** Nothing to disclose.

### T257. Different Brain States Relate to Opioid Versus Cocaine Use in Poly-Addicted Individuals

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**Background:** Functional connectivity analyses have traditionally utilized resting state data. However, recent data demonstrate that manipulation of brain state via task performance is optimal for amplification of individual differences in connectivity, particularly within the context of brain-behavior modeling. We have previously used connectome-based predictive modeling (CPM) — a data-driven method of identifying neural networks subserving specific behaviors — of reward task data to identify a network related to subsequent cocaine use. Here, we test (a) whether this network also relates to cocaine use during a different brain state (cognitive task performance) and (b) whether functional connectivity across brain states also relates to opioid use in the same sample of poly-addicted individuals.

**Methods:** Seventy-four individuals with co-occurring opioid and cocaine use participated in neuroimaging at the start of a 12-week treatment trial. Following exclusion for motion controls, fMRI data from 53 individuals were entered into CPM analyses to identify neural networks related to within-treatment abstinence. Separate CPMs were run to identify neural networks related to future cocaine and opioid use using reward and cognitive control task data. CPMs were run using leave-one-out-cross-validation and significance was determined using permutation-based testing, as

in prior work. Cocaine and opioid use during treatment were determined based on results of biweekly urine toxicology testing.

**Results:** CPM of reward task data identified networks associated with future cocaine use ($p = .001$) that were unrelated to future opioid use ($p = .30$). In contrast, CPM of cognitive data successfully identified networks associated with future opioid use ($p = .018$) that were unrelated to future cocaine use ($p = .75$). Consistent with other CPM work, identified networks for both analyses were complex and included both cortical and subcortical connections. Despite this complexity, the spatial extent of both cocaine and opioid networks together included only 1,743 edges (529 cocaine, 1,214 opioid), or less than 5% of possible connections. Spatial overlap between networks was minimal and included only 8 edges ($< .05\%$ of possible connections). Both networks were robust and were not significantly altered in follow-up analyses controlling for other baseline clinical variables, including treatment assignment or methadone dose.

**Conclusions:** These data indicate dissociable neural networks of cocaine versus opioid use in poly-addicted individuals and demonstrate the importance of considering brain state when building brain-behavior models. These findings support the existence of distinct neurobiological features subserving opioid and cocaine use, which may have important implications for improving existing addiction treatments, particularly in poly-addicted individuals.

**Keywords:** Abstinence, Connectome, Opioid Addiction, Cocaine Addiction

**Disclosure:** Nothing to disclose.

### T258. Elucidating the Effect of a Brief Motivational Drinking Intervention Using Neuroimaging: A Preliminary Study

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**Background:** Brief motivational interventions have empirical support for acutely reducing alcohol use among non-treatment seeking heavy drinkers. Neuroimaging techniques allow for the examination of the neurobiological effect of behavioral interventions, probing brain systems putatively involved in clinical response to treatment. Further, neurobiological circuits identified using fMRI can be used to predict treatment and drinking outcomes, providing unique information beyond that of self-report and behavior. While initial evidence indicates that psychological interventions are effective at reducing mesocorticolimbic response to alcohol-associated cues (Feldstein-Ewing et al., 2011), few studies have prospectively evaluated if psychosocial interventions attenuate neural cue-reactivity that in turn reduces drinking in the same population. Therefore, this study aimed to examine the effect of a brief motivational intervention on drinking outcomes, neural alcohol cue-reactivity, and the ability of neural alcohol cue-reactivity to predict drinking outcomes.

**Methods:** Non-treatment-seeking heavy drinking participants were randomized to receive a brief motivational interview intervention ($n = 22$; 13 M/9 F; average age $= 36.41$) or an attention-matched control ($n = 24$; 15 M/9 F; average age $=$ 32.29). The brief intervention consisted of a 30-45-minute individual face-to-face session offering personalized feedback and a menu of change options regarding alcohol use. The attention-matched control consisted of a 30-minute video about astronomy. Immediately following the intervention or control, participants underwent an fMRI scan where they completed an alcohol taste cues paradigm (modified from Filbey et al., 2008). Approximately four-weeks after the intervention (or control), participants completed a follow-up visit to report on their past-month drinking. Baseline and follow-up percent heavy drinking days (PHDD; HDD defined as $\geq 5$ drinks for men, $\geq 4$ drinks for women) was calculated for each participant. FMRIB's Software Library (FSL 5.0) was used for the neuroimaging analysis.

**Results:** Mixed models analysis revealed no significant effect of the brief motivational intervention on percent heavy drinking days compared to the control group ($\beta = -0.05$, $SE = 0.07$, $p = 0.48$). There was no significant effect of the brief motivational interview intervention on modulating neural activation to alcohol taste cues relative to water taste cues. There was, however, a significant indirect mediation of neural response to alcohol taste cues and PHDD, for both groups ($Z > 2.3$, $p < 0.05$), such that individuals who had greater neural reactivity to alcohol taste cues in the precuneus and prefrontal cortex had fewer PHDD in the four weeks following the fMRI visit.

**Conclusions:** A brief motivational interview was not more effective than a no-intervention condition in reducing percent heavy drinking days or modulating neural activation to alcohol taste cues in this sample of non-treatment seeking heavy drinkers. Across both groups, neural reactivity to alcohol cues in the precuneus and prefrontal cortex predicted decreased heavy drinking in the month following the fMRI scan. While participants in the control condition did not receive counseling on their drinking, they did elect to participate in a study about drinking and completed assessments regarding their drinking behavior. Therefore, participants across groups who had greater neural activation may have found the alcohol taste cues more salient, and reduced their heavy drinking following participation in the study visit.

**Keywords:** Brief Motivational Intervention, Functional MRI (fMRI), Binge Drinking, Alcohol and Substance Use Disorders

**Disclosure:** Nothing to disclose.

### T259. Brain Adaptations for Buprenorphine Medicated Postpartum Women

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**Background:** The rate of pregnant women addicted to opioids in the U.S. quadrupled in the past 15 years. For those opioid-addicted peripartum women who receive the gold standard treatment of buprenorphine medication (BM) to prevent withdrawal and miscarriage, 33% or more of affected new mothers' relapse to substance uses that may dysregulate stress system and interfere with parental sensitivity. Maternal behaviors are known to be governed by evolutionary conserved Maternal Behavior Neurocircuit (MBN) to regulate maternal caring and defensive behaviors, which are normally under reciprocal regulation. However, data on MBN adaptation in the early postpartum among BM mothers is lacking. The importance of addressing this knowledge gap is magnified by the transgenerational risks for mother-infant dyads affected by opioids. For this longitudinal magnetic resonance imaging study, we hypothesize that MBN physiology adapts in early postpartum as a function of buprenorphine treatment, mood, and parenting stress.

**Methods:** We studied 79 mothers in early PP, 42 of which completed structural and functional magnetic resonance imaging (MRI) scans at two time-points: T1 (1-month PP) and T2 (4 months PP). The participants reported depression symptoms and stress using Beck Depression Inventory and parenting stress index respectively at T1 & T2. We divided the participants into three groups: BM treated (BM, $n = 11$), non-opioid depressed control

(DC, n = 12), and non-opioid non-depressed healthy controls (HC, n = 19). At each timepoint, the participants underwent functional magnetic resonance imaging (fMRI) scans while performing 3 tasks: a baby-cry task, wherein subjects listened to own and other's baby cry and respective control sounds in a block design (30 s per block, 5 blocks per condition); a baby-face mirroring task, wherein subjects were asked to join (empathic mirroring) or observe (unresponsively observing) own or unknown child's face pictures of neutral, ambiguous, distressful, and joyful expressions - in a block design of four consecutive pictures (4 s/picture, one picture per emotional expression); and a resting state task of 8 minutes. The fMRI data were processed and analyzed in SPM 8.

**Results:** While BM mothers showed comorbid depressive mood symptoms similar to DC mothers, they exhibited even greater child-oriented worries than DC/HC mothers. Own vs. other's baby cry responses and relative brain volumes in the MBN are consistent with BM > DC/HC mothers brain physiology correlating with maternal worries. However, resting-state functional connectivity between MCN neurocircuits that mediate caregiving vs. defensive behaviors in animal models, was not antagonistic for BM mothers as among DC/HC mothers. The Child Face Task identifies the dorsomedial prefrontal cortex as differentially deactivated for emotional attunement to child's feelings as opposed to distantly observing (Join vs Observe, Own vs Other Child).

**Conclusions:** During the early postpartum, Buprenorphine Medication (BM) influences the early postpartum, adaptation of maternal brain and behaviors with BM mothers reporting depression symptoms and increased worries about their baby. BM appears to increase maternal behavior neurocircuit (MCN) responses - including both caregiving and defense circuits. BM increases resting state functional connectivity (rs-FC) in the maternal caregiving circuit. However, BM also increases rs-FC between caregiving and defense neurocircuits, which are normally regulated in reciprocal opposition. Furthermore, evidence from the child face task suggests that the dmPFC may be hijacked by addiction – potentially dysregulating the MBN and leading to exacerbated parenting stress. Thus, BM treatment appears to drive maternal brain physiology toward maternal behavior, consistent with the benefits of treatment – yet may also pose risks for exacerbated postpartum stress and reduced maternal sensitivity - suggesting translational recommendations.

**Keywords:** Brain Stress, Maternal Behavior, Postpartum Depression, Buprenorphine, Opioid Treatment

**Disclosure:** Nothing to disclose.

### T260. Alcohol Expectancy and Cerebral Responses to Cue-Elicited Craving in Adult Non-Dependent Drinkers

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**Background:** Along with an impaired ability to control urges to drink, craving is a hallmark of alcohol abuse and dependence. It is well known that alcohol-related cues evoke craving and expectations of positive outcomes associated with drinking. Alcohol expectancy (AE) represents subjective beliefs about the extent to which drinking will lead to positive outcomes (e.g., 'drinking makes me feel good;" 'alcohol makes me worry less").

Alcohol expectancy (AE) contributes to excessive drinking. Many imaging studies have examined cerebral responses to alcohol cues and how these regional processes related to problem drinking. However, it remains unclear how AE relates to cue response and whether cue response mediates the relationship between AE and problem drinking.

**Methods:** Sixty-one non-dependent drinkers (30.7 + /- 8.4 years, 33 men) were assessed with the Alcohol Expectancy Questionnaire (AEQ-3) and Alcohol Use Disorder Identification Test (AUDIT; 10.6 + /- 10.3) and underwent fMRI while exposed to alcohol and neutral cues in alternating blocks. Imaging data were processed with published routines. In whole-brain linear regression, we identified regional activations to alcohol vs. neutral cues in association with individual variation in AE. We employed generalized psychophysiological interactions (gPPI) to examine regional connectivity in association with AE. Further, we conducted mediation analyses to examine the inter-relationships between AE score, AUDIT score, and regional responses to alcohol vs. neutral cues.

**Results:** Alcohol as compared to neutral cue elicited higher craving rating ($3.1 \pm 2.5$ vs. $1.9 \pm 2.1$, $p < 0.0001$, paired t test). Alcohol but not neutral cue elicited craving was also correlated positively with AE score across subjects ($r = 0.43$, $p < 0.0006$; and $r = 0.16$, $p = 0.2141$, respectively).

At a corrected threshold, alcohol as compared to neutral cues engaged the occipital, retrosplenial, and medial orbitofrontal cortex as well as the left caudate head and the red nucleus. Bilateral thalamus showed a significant correlation in cue response and in left superior frontal cortical connectivity with AE score in a linear regression.

We used bilateral thalamus clusters as a seed region in gPPI analysis. The results showed a number of cortical and subcortical regions with higher interaction with the thalamus during alcohol vs. neutral cue blocks. Of these clusters, the gPPI effect size of the left superior frontal sulcus showed a positive correlation both with the AE ($r = 0.38$, $p = 0.0023$) and AUDIT ($r = 0.39$, $p = 0.0022$) score.

Finally, mediation analyses showed that AE score completely mediated the relationship between thalamic cue activity as well as superior frontal cortical connectivity and AUDIT score. The alternative models that AE led to problem drinking and, in turn, altered thalamic cue activity and connectivity were not supported.

**Conclusions:** In conclusion, the current study demonstrated the cue-related neural correlates of thalamic activity and connectivity in association with alcohol expectancy. To our knowledge these findings are the first to relate alcohol expectancy to cue elicited brain responses. These neural measures mediated the relationship between alcohol expectancy and severity of problem drinking and provide new markers of alcohol misuse.

**Keywords:** Alcohol, Cue Reactivity, Expectation Bias, Functional MRI (fMRI), Thalamus

**Disclosure:** Nothing to disclose.

### T261. Paired Associative Stimulation: A Novel TMS Method for Modifying Cognitive Networks

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**Background:** Cortical paired associative stimulation (cPAS) is a form of transcranial magnetic stimulation in which repeated paired pulses can induce plasticity at cortical synapses. cPAS has previously been used to modify outcomes in the motor domain. Here we used cPAS within the cognitive domain to modify behavioural outputs of response inhibition, goal-directed control and working memory.

**Methods:** In two separate studies we used cPAS targeting (i) the pre-supplementary motor area (pre-SMA) and right inferior

frontal cortex (rlFC) using -10, -4, + 4, + 10 msec intervals to modify the stop signal reaction task (N = 25); and (ii) the right intra parietal sulcus and right dorsolateral prefrontal cortex (rdlPFC) using -10, + 10, + /100 msec to modify goal-directed control and working memory (N = 30) healthy volunteers respectively. The target coordinates were selected using coordinates from meta-analyses of functional MRI tasks. The conditions were randomly ordered and separated by at least a week.

**Results:** Study 1: The stop signal reaction time showed a main effect of cPAS condition as a function of age (repeated measures ANOVA $F(3,57) = 4.05$, $p = 0.01$) relative to baseline. Younger subjects had impaired response inhibition when the pre-SMA pulse preceded the IFC pulse by 10 msec. In older individuals, response inhibition improved when the IFC pulse preceded the pre-SMA pulse by 4 msec. Study 2: Goal-directed control (Wilcoxon signed-rank test, $p = 0.015$, medium effect size $r = 0.284$) and the precision of working memory improved when the parietal pulse preceded the rdlPFC pulse by 10 msec relative to + /-100 msec but the two parameters were unrelated. There was no effect on delay discounting in either study.

**Conclusions:** We show for the first time that cPAS can modify cognitive networks such as response inhibition and goal-directed control presumably either through cortico-cortical or cortico-subcortical plasticity. These findings have implications for novel therapeutic interventions for impaired cognition in psychiatric disorders.

**Keywords:** TMS, Impulsivity, Goal-Directed Control, Response Inhibition, Working Memory

**Disclosure:** Nothing to disclose.

### T262. Relationship of PTSD and Recovery in a Sample of Dually Diagnosed Individuals on Medication Assisted Treatment

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**Background:** Experimental induction of "opioid-mediated stress-induced analgesia" has been shown in individuals with posttraumatic stress disorder (PTSD) (Pitman et al., 1990). Because of the involvement of opioid systems in PTSD stress response we wondered if PTSD would influence the outcome of those with opioid use disorder being treated with medication assisted therapy. Therefore, we are conducting an analysis to determine if PTSD may influence the recovery outcomes in those with opioid use disorder being treated with buprenorphine/naloxone therapy as compared with those without PTSD.

**Methods:** Participants were a group of 27 dually diagnosed individuals receiving buprenorphine/naloxone and group therapy through a university mental health center as part of study evaluating supported employment. Approximately 30% of these participants had a posttraumatic stress disorder (PTSD) diagnosis. Their adherence to BUP/NAL treatment was monitored through weekly drug screen tests and attendance to treatment.

**Results:** Of the thirteen participants that had a PTSD diagnosis, 9 (69%) stayed in treatment for at least 12 months as compared with 4 participants (30%) who left treatment for their opioid use disorder 8 months or less. Four of the 10 participants who remained in treatment for at least 24 months had PTSD.

**Conclusions:** Preliminary finding of higher retention needs confirmation in a larger sample. PTSD is common among those with opioid use disorders. Whether PTSD influences the outcome of recovery in opioid use disorder has theoretical and clinical implications. Larger studies evaluating outcomes of those with PTSD and opioid use disorder may be helpful in understanding short term and long-term recovery.

**Keywords:** PTSD, Opioid Use Disorder, Outcome

**Disclosure:** Nothing to disclose.

### T263. Repeated Δ9-tetrahydrocannabinol (THC of Marijuana) Administration Promotes Dramatic Changes in Dopamine Signaling Parameters in Basal Ganglia: THC Combined With Cannabidiol Does Not

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**Background:** Regular heavy marijuana use in susceptible populations is associated with heightened risks for marijuana (cannabis) use disorder, memory impairment, and less frequently, psychiatric disorders, including schizophrenia, bipolar disorder, depression, social anxiety, and exacerbation of psychiatric symptoms. Of the 100 or more cannabinoids in the marijuana plant, THC is the most abundant cannabinoid followed by cannabidiol (CBD). The ratio of THC:CBD is rising rapidly in commercial marijuana. In humans, consumption of marijuana with high THC:CBD ratios are associated with heightened euphoria, anxiogenesis, and psychotic symptoms, whereas low THC:CBD ratios are linked to sedation and attenuation of psychosis, anxiety and cognitive deficits. With increasing evidence for a causal relationship between long-term potent marijuana use and psychiatric symptoms, we sought to determine if repeated administration of THC or THC combined with CBD engendered different biochemical responses in post-mortem brain of nonhuman primate, that could reveal contrasting molecular effects underlying their different pharmacological responses. We previously reported that THC elevated expression of dcc mRNA and the D1-D2 dopamine receptor heteromer. The dcc gene encodes DCC, an axonal guidance molecule critical for formation of prefrontal cortical dopamine circuits during adolescence and is increasingly implicated in major psychiatric disorders. The dopamine D1-D2 receptor heteromer complex is a regulator of rewarding or aversive effects. Notably we discovered that CBD, if combined with THC, attenuated dcc up-regulation and increased formation of the D1-D2 dopamine receptor heteromer. Our present goals were to determine whether downstream elements of dopamine signaling were altered by repeated administration of THC to adult monkeys and whether CBD modulated the neurochemical effects of THC.

**Methods:** Adult rhesus monkeys (Macaca mulatta) were treated for 24 days with escalating doses of THC, or THC combined with CBD, or vehicle control (n = 3/group). THC was administered during 24 total study days in a range of doses 0.1-3.2 mg/kg, i.m.) and brains were harvested on day 25. THC + CBD were administered for 24 total study days for THC (doses ranging from 0.1-3.2 mg/kg. i/m/) and CBD (days 6-24; doses ranging from 1-3 mg/kg i.m.), with brain harvest on day 25. Gene expression levels were measured following mRNA isolation from various brain regions. Protein assays were performed by Western blots using selective antibodies

**Results:** In nonhuman primates treated daily for 24 days, repeated THC administration significantly increased: (1) D1 receptor mRNA expression, but not D2 receptor or dopamine transporter mRNA expression in the caudate nucleus; this effect was abolished if THC and CBD were administered together; (2) colocalization of Substance P and A2AR in neurons, possibly indicating that a number of medium spiny neurons had adopted a different phenotype which coexpressed the D1 and D2 receptor, Substance P, adenosine A2A receptor. CBD co-treatment inhibited

this effect; (3) pCaMKIIα more than 50% over basal phosphorylation in vehicle-treated monkeys, an effect abolished with co-administration with CBD; (4) phosphorylation of Thr75-DARPP-32 over basal levels in caudate and NAcc, but the effect was attenuated by CBD co-treatment. The protein DARPP-32 is involved in modulation of cAMP signaling in striatal neurons; (5) the number of neurons expressing the Thr-75 phosphorylated form of DARPP-32, in line with the dramatic increase in D1-D2 heteromer, CBD reduced the THC-induced increase. (6) ΔFosB; CBD abolished this effect in the NAc and CN when co-administered with THC. Other contrasting effects of THC alone or THC combined with CBD were also observed.

**Conclusions:** These exciting discoveries reveal that THC induced numerous changes in the dopamine and cannabinoid signaling pathways, which were antagonized by the co-administration of CBD. Although the full mechanisms underlying THC and CBD effects remain to be elucidated, it is clear that current strains of marijuana bred to produce high THC:CBD ratios may result in unintended neuroadaptive consequences. Our results are relevant to candidate cannabinoid therapeutics and to regulatory oversight of the ratio of THC:CBD in strains of marijuana.

**Keywords:** Marijuana, Dopamine, Cannabidiol, THC, Marijuana Policy

**Disclosure:** Nothing to disclose.

### T264. Epigenetic Factors Mediate the Increase in Sensitivity of Ethanol Reward in the Central Nucleus of the Amygdala Produced by Adolescent Binge Ethanol Exposure in Male, but Not Female Wistar Rats

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**Background:** Adolescent binge drinking produces neuroadaptations that are thought to predispose individuals to develop alcohol use disorder (AUD) during adulthood. Past research has indicated that alterations in epigenetic factors produced by adolescent binge drinking may be a critical component of the neuroadaptations observed in adulthood. The central amygdala (CeA) is one area of interests to examine the alcohol intermittent exposure (AIE) effects of epigenetics factors in adulthood. Previous research has demonstrated that the CeA mediates alcohol addiction behaviors and there are sex differences in the CeA in response to EtOH. In addition, AIE results in epigenetic perturbations within the CeA. Our lab has found animals will intracranially self-administer (ICSA) EtOH directly into the CeA and that AIE increases the sensitivity of the CeA to EtOH in adulthood.

**Methods:** The current experiments were conducted to determine if there are sex differences in the effects of adolescent EtOH exposure on EtOH reinforcement behaviors in the CeA that may be mediated by epigenetic factors. Briefly, rats were treated with (i.g.) 4 g/kg EtOH (25% v/v) 2 days on/2days off during adolescence (PND 30-55). The H2O group received comparable treatment with water. At PND 90, the animals underwent surgery targeting the CeA. After the recovery period, the rats were pretreated with 22 mM/0.5ul of histone deacetylase (HDAC) inhibitor Trichostatin A (TSA) during the acquisition phase of ICSA and during all test days the rats received TSA (3 h) prior to EtOH ICSA sessions. The ICSA session were 4 h and conducted every other day for 7 sessions.

**Results:** The data indicated that pretreatment with TSA in the CeA attenuated acquisition and reinstatement of EtOH reinforcement in males Wistar rats but had no effect on extinction. In contrast, pretreatment with TSA in the CeA did not alter acquisition, extinction, or reinstatement of EtOH reinforcement in female Wistar rats.

**Conclusions:** Overall, the data indicated the effects of adolescent EtOH exposure on reinforcement in the CeA is mediated by epigenetic factors in male Wistar rats but not female Wistar rats.

**Keywords:** Adolescent Alcohol, Epigenetic, Reinforcement, Central Amygdala, Intracranial Self-Administration

**Disclosure:** Nothing to disclose.

### T265. Microinjections of Ethanol and Nicotine, but Not Ethanol or Nicotine, Into the Posterior VTA Increases Glutamate Levels Within the Nucleus Accumbens Shell

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**Background:** The vast majority of individuals diagnosed with alcohol use disorder (AUD) concurrently smoke (65-89%). An individual with AUD is also more like to be determined to have a nicotine use disorder (NUD) more than 4 times often than individuals who do not have AUD. The interaction between alcohol and nicotine are numerous, but the research field has persisted in studying these compounds individually. Recent clinical and preclinical data has indicated that the standard pharmaceuticals for the treatment of AUD (naltrexone) and NUD (varenicline) are ineffective at reducing alcohol and nicotine co-administration. The lack of efficacy of these compounds may be predicted upon unique neuroadaptations produced by alcohol and nicotine co-abuse. Recent data indicate that alcohol and nicotine act synergistically in the posterior VTA; 1) to produce reward and 2) result in unique alterations in gene expression in the nucleus accumbens shell (AcbSh). Further research has indicated that EtOH and nicotine microinjection into the posterior VTA, but not equivalent administration of EtOH or nicotine, results in increase protein expression of brain derived neurotrophic factor (BDNF) in the AcbSh. Microinjection of EtOH and nicotine into the posterior VTA increases dopamine (DA) levels in the AcbSh. One subset of VTA DA neurons have been shown to co-release glutamate. The current experiment examined the effects of rewarding (threshold) or subthreshold doses of EtOH, nicotine, or EtOH + nicotine microinjected into the posterior VTA on DA and glutamate release within the nucleus accumbens shell (AcbSh)

**Methods:** In male Wistar rats, neurotransmitter levels were determined in the AcbSh following acute microinjections of artificial cerebrospinal fluid (aCSF), EtOH, nicotine or EtOH + nicotine. Animals received experimenter-controlled microinjections into the pVTA designed to emulate intracranial self-administration levels (5 second injections, 15 second timeout period, 100 nL/injection over 10 minutes), concurrently with in vivo microdialysis of the AcbSh. Rats were microinjected with aCSF, subthreshold (100 mg%) and threshold (150 mg%) EtOH, subthreshold (10 μM) or threshold (50 μM) nicotine, or combinations of subthreshold (100 mg% EtOH + 10 μM nicotine) or threshold (150 mg% EtOH + 50 μM nicotine) EtOH + nicotine.

**Results:** DA levels within the AcbSh increased by 35% following microinjections of EtOH or nicotine into the pVTA. EtOH + nicotine resulted in an 80% increase of DA release, which persisted over time compared to EtOH or nicotine alone. Microinjection of EtOH or nicotine alone had no effect on glutamate levels in the AcbSh.

In contrast, co-administration of EtOH + nicotine into the pVTA increased glutamate levels 40% in the AcbSh.

**Conclusions:** These data indicate that an increase in glutamate release within the AcbSh was not dependent upon an increase in DA. Results suggest that only co-administration of EtOH + nicotine activate pVTA DA/glutamate neurons projecting to the AcbSh. This specific activation could be part of the biological basis for previous work showing significant increases in BDNF levels within the AcbSh following co-administration of EtOH + nicotine into the pVTA. The findings reveal that only EtOH + nicotine co-administration/use results in distinct perturbations that could stimulate plasticity throughout the mesolimbic system and promote the development of addiction.

**Keywords:** Reward, Polydrug Use, Neurochemistry

**Disclosure:** Nothing to disclose.

### T266. Dopamine and Adenosine Receptors Differentially Modulate Methamphetamine Seeking

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**Background:** Methamphetamine (MA) dependence and addiction is a serious public health concern having significant adverse effects. To date, there are no known pharmacological therapies with established efficacy for the treatment of MA addiction. Dopamine and adenosine receptors are known to interact in the striatal areas for movement generation, and previous work has demonstrated their individual involvement in drug seeking behaviors. The present studies examine the interactive effects of dopamine and adenosine receptor subtypes in the reinstatement of extinguished MA seeking.

**Methods:** Male Sprague-Dawley rats (n = 5-14/group) were trained to lever press for MA in daily 2-hour self-administration sessions on a fixed-ratio 1 schedule for 12 consecutive days. After 1 day of abstinence, lever pressing was extinguished in daily extinction sessions. MA seeking was measured during a reinstatement test to test a variety of dopamine and adenosine receptor ligands. We first tested the sufficiency of dopamine receptor stimulation to induce MA seeking using systemic administration of agonists for dopamine D1 (SKF 81297, 0-1 mg/kg, sc), D2 (Sumanirole, 0-10 mg/kg, ip), D3 (7-OH-DPAT, 0-10 mg/kg, ip) and D4 (A412997, 0-10 mg/kg, ip) receptor subtypes. We next tested whether the dopamine subtype-selective agonists (SKF 81297 and 7-OH-DPAT) infused directly into the nucleus accumbens were sufficient to induce MA seeking. To evaluate the cooperative effects of the dopamine D1 and D3 receptor subtypes on MA seeking, we co-infused the D1 and D3 agonists at subthreshold doses into the nucleus accumbens. Lastly, adenosine A1 (CPA) and A2A (CGS 21680) receptor agonists were administered into the nucleus accumbens alone or in combination with dopamine D1 and D3 agonists to test for their sufficiency to induce MA seeking and modulate dopamine-induced MA seeking, respectively. ANOVAs were used to analyze lever responding during the reinstatement tests and significant main effects were evaluated by post-hoc comparisons.

**Results:** A dose-dependent increase in MA seeking was observed following systemic administration of the D1- or D3-selective agonist ($p < 0.001$). No effects of D2 or D4 receptor stimulation on MA seeking were observed. Stimulation of D1 ($p < 0.05$) or D3 ($p < 0.01$) receptors in the nucleus accumbens shell produced the most robust MA seeking response suggesting the nucleus accumbens shell as the primary locus of their effects on MA seeking. Simultaneous infusion of low, ineffective doses of the D1 and D3 agonists into the nucleus accumbens shell resulted in a significant increase in MA seeking suggestive of synergism between these two receptors ($p < 0.01$). Adenosine A1 receptor stimulation in the nucleus accumbens shell had no effect on MA seeking when administered alone, but significantly reduced MA seeking induced by either D1 or D3 receptor stimulation ($p < 0.001$). Adenosine A2A receptor stimulation in the nucleus accumbens shell had no effects on MA seeking.

**Conclusions:** These findings suggest that dopamine and adenosine receptors interact to both facilitate and impair MA seeking. In particular, dopamine D1 and D3 receptor stimulation appear to have synergistic effects on MA seeking, while adenosine A1 receptors appear to counteract D1- and D3-induced MA seeking. Together the results provide an enriched view of the mechanisms that contribute to MA seeking.

**Keywords:** Drug Relapse, Psychostimulant, Purine

**Disclosure:** Nothing to disclose.

### T267. Sex Differences in Opioid Reinforcement Under a Fentanyl Vs. Food Choice Procedure in Rats

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**Background:** Sex differences in opioid preclinical pharmacology have been observed on endpoints related to opioid abuse and opioid use disorder. The aim of the present study was to examine potential sex differences in opioid reinforcement in rats using a novel fentanyl vs. liquid food choice procedure. Because choice procedures provide a measure of relative reinforcement that depends upon not only the self-administered drug, but also the magnitude and type of alternative nondrug reinforcer, two behavioral economic endpoints were initially determined for both fentanyl and liquid food in male and female rats.

**Methods:** 12 Sprague-Dawley rats (6 male, 6 female) were acquired at 10 weeks of age (Envigo Laboratories, New Jersey, USA) and surgically implanted with custom jugular catheters and vascular access ports (Instech, Plymouth Meeting, PA). Animals were singly housed in a vivarium maintained on a 12-h light/dark cycle (lights off at 6:00 PM). Water and food were provided ad lib in the home cage. Behavioral sessions were conducted 5-7 days per week at approximately 2:00 PM - 4:00 PM. Animal maintenance and research were conducted in accordance with the 2011 guidelines of the NIH Committee on Laboratory Animal Resources and protocols were approved by the Institutional Animal Care and Use Committee. Experiment one examined sex differences in fentanyl reinforcement using behavioral economic procedures. Rats were trained to respond under a fixed-ratio (FR) 5 / time out 20 s procedure and FR5 completion resulted in a 5-s presentation of (18% or 56%) liquid food. Once behavior was stable, the FR was decreased to one until the number of liquid food presentations earned was □ 20% of the last three session mean with no trends. Subsequently, the FR (i.e., 1, 3, 6, 10, 18, 32, 56, 100, 180, 320, 560, 1000) increased across consecutive sessions until a rat failed to complete the response requirement during the session. After both liquid food concentrations were evaluated, rats were then implanted with an intravenous catheter, and experimental procedures were repeated as described above for two fentanyl doses (3.2 and then 10 μg/kg/inj). Experiment 2 examined sex differences in fentanyl reinforcement under a concurrent FR5: FR5 schedule of liquid food and fentanyl availability. The behavioral session consisted of five 20-min response components each preceded by a 4-min "sample" component. Each sample component started with a non-contingent injection of the unit fentanyl dose available during the subsequent response

component followed by a 2-min time out. Next, a 5-s presentation of liquid food was programmed followed by a 2-min time out. Following this second time out, the response component began. During each response component, both levers were extended, a red stimulus light above the left lever was illuminated to signal liquid food availability and a green stimulus light above the right lever was illuminated to signal fentanyl availability. FR5 completion on the left lever resulted in a 5-s presentation of liquid food; whereas, FR5 completion on the right lever resulted in fentanyl delivery. Responding on one lever reset the ratio requirement for the other lever. A different fentanyl dose was available during each of the five successive response components (0, 0.32, 1.0, 3.2, and 10 µg/kg/inj during components 1-5, respectively). Fentanyl dose varied by changing the infusion duration (300 g rat; 0, 0.5, 1.56, 5, and 15.6-s of pump activation during components 1-5, respectively) and visually signaled by the frequency of the flashing right green light above the drug-associated lever in 3-s cycles. Choice training was considered stable when the smallest fentanyl dose that maintained at least 80% of completed ratio requirements on the fentanyl-associated lever was non-trending for three consecutive days. Fentanyl vs. food choice dose-effect functions were determined under both 18% and 56% liquid food availability.

**Results:** Liquid food demand was greater in females (18% liquid food: [$F(1,13) = 20$, $p = 0.0006$; 56% liquid food: [$F(1,13) = 6.5$, $p = 0.024$]). Both essential and derived Q0 values did not differ between sexes. Fentanyl demand was also greater in females (3.2 µg/kg/inj: [$F(1,17) = 15$; $p = 0.0012$], 10 µg/kg/inj: [$F(1,19) = 17$; $p = 0.0005$]). Essential value was greater in females only for 10 µg/kg/inj fentanyl (males $M = 23.0$, $SD = 13.9$; females $M = 51.5$, $SD = 20.3$; [$t(2.84) = 10$; $p = 0.02$]). Q0 values did not differ between sexes. For experiment 2, rats primarily chose food when fentanyl was not available (0 µg/kg/inj) or the unit fentanyl dose was small (0.32-1 µg/kg/inj). Furthermore, behavior was allocated towards fentanyl when larger fentanyl doses were available (3.2-10 µg/kg/inj), irrespective of the liquid food concentration. Males chose a greater percentage of 3.2 µg/kg/inj fentanyl injections over 18% liquid food than females (Dose: [$F(4,32) = 90.7$; $p < 0.0001$]; Interaction: [$F(4,32) = 3.6$; $p = 0.015$]; Sex: [$F(1,8) = 3.7$; $p = 0.085$]). Percent fentanyl choice was not different between sexes during 56% liquid food availability.

**Conclusions:** These results demonstrate significant sex differences in opioid reinforcement under certain experimental conditions. Moreover, these results provide an empirical foundation to probe both pharmacological and neurobiological mechanisms of opioid reinforcement.

**Keywords:** Opioid Abuse, Self-Administration, Sex Difference, Fentanyl, Choice Procedure

**Disclosure:** Nothing to disclose.

### T268. Early Life Stress-Potentiated Cocaine Seeking is Mediated by Dysregulation of the Interaction Between Kappa Opioid Receptors and Dopamine Transporters

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**Background:** Cocaine addiction exhibits a complex pathology, which is often exacerbated in individuals exposed to major adverse events during their life. Particularly, exposure to chronic stress during adolescence augments drug- and cue-induced cocaine craving as well as severity of withdrawal symptoms, potentially driving cocaine addiction in humans. Similarly, in rodents, adolescent social isolation (aSI) results in augmented locomotor response to systemic cocaine administration and elevated cocaine seeking compared to rats that are group housed during adolescence (aGH). Stress results in an increase in dynorphin, the endogenous kappa opioid receptor (KOR) agonist. Moreover, aSI results in upregulation of KOR binding sites and function. Additionally, KOR activation time-dependently modulates vulnerability to cocaine and reward processing. Thus, the goal of this study was to compare the reinforcing efficacy of cocaine in aSI and aGH animals and to investigate the underlying mechanisms - interaction between KORs and dopamine transporters - involved in driving the potentiated effect of cocaine on locomotor behavior and augmented cocaine seeking in aSI rats.

**Methods:** Male Long Evans rats were either housed in groups (4 rats/cage) or individually (1 rat/cage) for six weeks (PD 28 – 74). After reaching adulthood, experiments were conducted to examine effects of acute cocaine (15 mg/kg) on locomotion and dopamine levels in the nucleus accumbens (NAc) using in vivo microdialysis in freely moving rats. Cocaine intake (fixed ratio schedule of reinforcement using long access - 6-hour daily sessions) and seeking (using progresive ratio schedule of reinforcement and behavioral economics) was examined in self-administering aGH and aSI rats. KOR function and cocaine potency at the dopamine transporter were examined using ex vivo voltammetry. Briefly, cumulative concentrations of U50,488 (30 – 1000 nM), a KOR agonist, were bath applied to accumbal slices from cocaine exposed and cocaine naive aGH and aSI rats and electrically evoked dopamine release was measured from NAc. Cumulative concentrations of cocaine (0.03 - 30 uM) were bath applied to NAc containing slices and cocaine's ability to inhibit the dopamine transporter was measured. Finally, KOR and dopamine transporter interaction was examined by inhibiting the KORs and overexpressing KORs (using a Cre dependent KOR overexpressing virus in TH:Cre positive aGH rats) in two separate experiments. Cocaine potency at the dopamine transport was examined in both.

**Results:** Systemic administration of cocaine (15 mg/kg; i.p.) resulted in an augmented locomotor response to cocaine. Self-administration using a progressive ratio schedule showed an enhanced motivation to self-administer cocaine in aSI compared to aGH rats (mean final ratio: aGH, 77; aSI, 268; at 2.25 mg/kg/infusion). Threshold analysis, during which the cocaine dose administered for each lever press is systematically reduced in ten-minute bins, showed that rats exposed to aSI were willing to "pay a greater price" to maintain their level of cocaine intake compared to aGH (lever presses/mg of cocaine: aGH, 200; aSI, 500). Administration of nor-binaltorphimine (nBNI; 10 mg/kg; i.p.), a KOR antagonist reduced this enhanced motivation to self-administer cocaine selectively in aSI rats (lever presses/mg of cocaine post nBNI: aGH, 198; aSI, 210). Interestingly, cocaine consumption in the two groups was not different. Voltammetry studies revealed that KOR function was augmented in cocaine exposed aGH rats, and cocaine naive and exposed aSI rats compared to cocaine naive aGH rats. Cocaine potency was increased in cocaine naive and exposed aSI rats compared to cocaine naive aGH rats; cocaine potency was reduced in cocaine exposed aGH rats. Surprisingly, cocaine exposure did not alter KOR function or cocaine potency in aSI rats - perhaps due to a ceiling effect. nBNI pre-treatment increased cocaine potency in cocaine exposed aGH rats and attenuated it in cocaine exposed and naive aSI rats.

**Conclusions:** Together, these data suggest that the reinforcing value of cocaine is greater for rats exposed to early-life stress than the control group as there is no difference in intake when the cost of self-administering cocaine is only a single lever-press. It is likely that rats exposed to chronic early-life stress self-administer cocaine for positive reinforcement, thus increasing the risk of developing addictive behaviors. The KOR inhibition-induced attenuation of cocaine seeking suggests that KORs potentially contribute to the augmented motivation to self-administer cocaine. Voltammetry studies indicate that both chronic stress

and chronic cocaine exposure (specifically in stress naive rats) results in increased KOR function. This potentiation in KOR function alters dopamine transporter function, albiet in separate ways. However, KOR blockade corrects the alteration in both aSI and cocaine exposed aGH rats. Thus, the reversal in cocaine-seeking behavior following KOR inhibition in aSI is likely driven by treating the dysregulated interaction between KORs and dopamine transporters. Therefore, KORs are promising therapeutic targets to treat cocaine addiction especially in individuals exposed to childhood adversities and are therefore predisposed to cocaine addiction.

**Keywords:** Cocaine Seeking, Kappa Opioid Receptors, Dopamine Transporter

**Disclosure:** Nothing to disclose.

### T269. Long-Term Inhibition of mu Opioid Receptor Function In Vitro and In Vivo by Methocinnamox (MCAM), a Long Acting mu Opioid Receptor Antagonist

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**Background:** An opioid epidemic is currently raging in the United States with over 115 deaths daily due to opioid overdose. The increasing availability of very potent opioids, which can be lethal when taken in even small doses, and the frequent use of opioids in combination with sedative drugs such as benzodiazepines, has contributed to the dramatic rise in opioid overdose deaths. Current pharmacological treatment of opioid overdose relies exclusively on the competitive antagonist naloxone (Narcan) that has a relatively short duration of action. Naloxone, given either by injection or intranasally, can reverse completely the respiratory depression and profound sedation produced by opioid agonists, however, naloxone's effectiveness is limited if large doses of a potent opioid (e.g., fentanyl) have been taken. MCAM was synthesized in the late 1900s as an antagonist for the mu opioid receptor, the target for opioids like morphine, heroin and fentanyl. Broadbear et al., (J Pharmacol Exp Ther, 294:933-940, 2000) reported that MCAM could attenuate the antinociceptive effects of morphine in mice for more than 48 h. We hypothesize that MCAM, due to its unusual pharmacological characteristics including a long duration of action, is better than currently available treatments to reverse opioid agonist overdose and to prevent the actions of opioid agonists that lead to abuse and dependence. Here we characterized the antagonist properties of MCAM at mu, delta and kappa opioid receptors. We also compared the effects of MCAM to that of naloxone at mu receptors.

**Methods:** Using HEK cell lines that stably express either human mu, delta or kappa opioid receptors, along with the bioluminescent cAMP sensor, Glosensor (Promega), we tested the effects of MCAM and the opioid receptor antagonist, naloxone, on opioid receptor agonist concentration response curves for the inhibition of forskolin-stimulated cAMP levels in response to either DAMGO (mu agonist), DPDPE (delta agonist) or U50488 (kappa agonist). We also measured the effect of MCAM to antagonize peripheral opioid receptor-mediated antinociception in the rat hindpaw. Following intraplantar (i.pl.) injections of MCAM or vehicle, thresholds for paw withdrawal from a heat stimulus were measured after i.pl. injection of PGE2 with either vehicle, DAMGO or U50488.

**Results:** Incubation with DAMGO inhibited forskolin-mediated stimulation of cyclic AMP levels by 40 ± 2 % with an EC50 concentration of 5 nM. Pretreatment with MCAM (10 nM, 10x reported Ki) for 15 min antagonized the DAMGO response, shifting the concentration response curve (CRC) to DAMGO to the right (1000-fold) along with suppression of the maximal response by 50%. Treatment with MCAM alone had no effect on either basal cAMP levels or forskolin-stimulated cAMP levels. The magnitude of MCAM's antagonist effect increased following a 2 h pretreatment. Pretreatment with naloxone (NLX, 100 nM, 10 x Ki) for 15 min also antagonized DAMGO-mediated inhibition of stimulated cAMP levels. However, as expected for a competitive antagonist and by contrast to MCAM, the 10-fold shift in the DAMGO CRC by NLX was fully surmountable and there was no further antagonist effect with 2 h of NLX pretreatment. We next compared the persistence of antagonism by MCAM to that produced by naloxone. MCAM-mediated antagonism following a 2 h pretreatment was unchanged after a rigorous washout period whereas by contrast, antagonism produced by NLX was fully reversible. We also tested the effects of pretreatment with MCAM for 15 min and 2 h on CRCs to the delta and kappa agonists. Following pretreatment with MCAM (20 nM, 10x reported Ki at delta) for either 15 m or 2 h, the CRC to the delta agonist, DPDPE was shifted ~ 10-fold to the right with no change in the maximal response. Similarly, the CRC to the kappa agonist, U50488, was shifted ~ 10-fold to the right with no change in the maximal response (i.e., fully surmountable) following pretreatment with MCAM (50 nM, 10x reported Ki at kappa) for either 15 m or 2 h. Further, antagonism produced by MCAM was fully reversible at both delta and kappa receptors. In vivo, intraplantar (i.pl.) injection of either DAMGO (20 µg/50 µl i.pl.) or U50488 (0.1 µg/50 µl i.pl.) produced a significant reduction in PGE2-evoked thermal allodynia. Pretreatment with MCAM at 10 nM (0.25 µg/50 µl i.pl) or 50 nM (1.25 µg/50 µl i.pl) 15 min prior to PGE2 injection had no effect on PGE2-induced thermal hypersensitivity; however, significantly inhibited the antinociceptive response to either DAMGO or U50488 respectively. The DAMGO-mediated antinociception remained blocked 24 and 48 h after a single administration of MCAM. By contrast, when tested 24 h after administration, MCAM did not alter U504880-mediated antinociceptive responses.

**Conclusions:** The time-dependent nature of the antagonism, the depression of the maximal mu agonist response, the lack of reversibility following wash-out, and the fact that MCAM did not elicit long-term antagonist effects at either delta or kappa opioid receptors, suggests that MCAM may be a selective, long acting (perhaps irreversible) mu opioid receptor antagonist. Such a drug may be a better treatment for opioid overdose than naloxone as the long-term antagonism of mu opioid receptors by MCAM would prevent re-narcotization and would be unaffected by large doses of opioid agonist abused. This work was supported by a pilot grant to JHW and KAB by the Addiction Research, Treatment & Training (ARTT) Center of Excellence at UT Health San Antonio.

**Keywords:** Opioid Overdose, Opioid Receptors, Opioid Antagonist Treatment

**Disclosure:** Nothing to disclose.

### T270. GABABR Function Regulates Feedforward Inhibitory Microcircuits in the Nucleus Accumbens Core

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**Background:** Illicit drug use is a pervasive socioeconomic issue with complex neurobiological effects. While emphasis has been placed on synaptic adaptations at excitatory synapses in the nucleus accumbens (NAc), less is known how feedforward inhibitory microcircuitry contributes to drug-related behavioral

states. The primary cell-type in the NAc is the GABAergic medium spiny projection neuron (MSN). MSNs can be differentiation based on the expression of D1 or D2 dopamine receptor, with synaptic rearrangements at D1- and D2-expressing MSNs facilitating antagonistic motivational outcomes. Coordinated MSN output is entrained by feedforward inhibitory signaling mediated by parvalbumin (PV)-expressing fast-spiking interneurons (INs). Feedforward inhibition in the NAc occurs as glutamatergic inputs onto D1 and D2 MSNs collateralize onto parvalbumin (PV)-expressing interneurons (INs), which in turn exert robust GABAergic control over MSN output. Recent evidence suggests that modulating synaptic strength within PV-IN-embedded microcircuits gates NAc-dependent motivational learning. The GABAB receptor (GABABR), a Gi/o G-protein-coupled receptor (GPCR), has been shown to modulate synaptic strength at short- and long timescales in various regions, including the NAc. Furthermore, pharmacotherapy targeting GABABR has shown promising therapeutic potential in pre-clinical and clinical studies of substance use disorder (SUD). Therefore, we hypothesized that GABABR activity modulates distinct synaptic elements within feedforward inhibitory architecture of the NAc core to elicit changes in overall circuit dynamics.

**Methods:** To test the hypothesis that GABABR modulates PV-IN-embedded feedforward inhibitory networks in the NAc core, we performed visualized whole-cell patch clamp electrophysiology in optogenetically-manipulated transgenic mice. Drd1a-tdTomato bacterial artificial chromosome (BAC) reporter mice were used to distinguish tdTomato-expressing D1(+) MSNs from D2 (i.e., D1(-)) MSNs. For a subset of experiments, PVCre mice, in which the expression of Cre is driven by the PV promoter, were crossed with Cre-inducible tdTomato mice (tdTomatofl/fl), enabling the identification of PV-INs. PV-IN-to-MSN synapses were examined by crossing PVCre mice were with conditional channel rhodopsin (ChR2) and Drd1a-tdTomato mice, enabling optically-evoked stimuli from PV-INs to be recorded from D1(+) and D1(-) MSNs. Input-specificity was obtained via stereotaxic delivering of a ChR2-containing AAV viral vector (AAV-ChR2-CaMKII-eYFP) into the prefrontal cortex (PFC) or mediodorsal thalamus (MDT).

**Results:** We demonstrate that presynaptic GABABR is tonically active at D1(+) MSN synapses and depresses glutamatergic synapses onto D1(+) and D1(-) MSNs by inhibiting voltage-gated Ca2+ channels (VGCCs). These mechanisms recruit intracellular effectors distinct from cannabinoid receptor type-1 (CB1), as GABABR activity fails to occlude the expression of depolarization-induced suppression of excitation (DSE), a CB1-dependent form of short-term plasticity. In contrast, GABABR activity depresses inhibitory transmission at PV-IN-to-D1(-) MSN synapses by activating presynaptic inward rectifying K+ channels (Kir). Although GABABR activity does not trigger inhibitory long-term depression (iLTD) at PV-IN-to-MSN synapses, we find that heterogeneous GABAergic inputs onto MSNs undergo GABABR-dependent LTD at D1(+) MSNs only. Utilizing viral-mediated gene transfer to target corticothalamic afferents to the NAc core, we found that GABABR-induced long-term depression (LTD) occurs presynaptically at PFC- and MDT-to-NAc synapses onto D1(+) and D1(-) MSNs, respectively. Finally, we report a novel regulatory role for PV-INs at glutamatergic synapses dependent on heterosynaptic crosstalk via GABABR. Optically-evoked stimuli from PV-INs at 30 Hz for 4-min, a frequency within dynamic range of PV-IN-directed gamma oscillations, depresses glutamatergic synapses onto D1(+) and D(-) MSNs. This effect is enhanced in the presence of a GABABR positive allosteric modulator and blocked by a GABABR antagonist.

**Conclusions:** These data point to a critical role for GABABR in regulating synaptic elements within PV-directed feedforward inhibitory circuits in the NAc core. While PV-IN-specific regulation of circuit activity has been demonstrated in various regions, we provide evidence of a novel form of heterosynaptic plasticity at glutamatergic synapses in the NAc mediated by GABABR. Understanding synaptic mechanisms by which NAc circuit function can be modulated will assist in the development future therapeutics for the treatment of SUD and SUD-related disorders.

**Keywords:** Nucleus Accumbens, Feed Forward Inhibition, Synaptic Plasticity, GABAB, Addiction Circuitry

**Disclosure:** Nothing to disclose.

### T271. Alteration in AMPA Receptor Subunit Expression and Receptor Binding Among Patients With Addictive Disorders: A Systematic Review of Human Postmortem Studies

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**Background:** Altered trafficking of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors has been reported in postmortem studies and suggested the involvement of AMPA receptors in the pathophysiology underpinning addictive disorders. However, these findings seemed mixed.

**Methods:** A systematic literature search was conducted, using PubMed and Embase (last search, May 2018), to identify human postmortem studies that examined the expression of proteins and mRNA of AMPA receptor subunits in patients with addictive disorders in comparison to healthy controls.

**Results:** Twelve (18 studies) out of 942 articles were identified to be relevant. Eight studies included alcohol use disorders (AUD) and four studies included heroin/cocaine abusers. The most frequently investigated regions were the hippocampus (3 studies), amygdala (3 studies), and putamen (3 studies). In summary, 2 out of the 3 studies showed an increase in the expression of AMPA receptors in the hippocampus while the other study found no change. Two studies to examine the amygdala demonstrated either a decreased or no change in receptor expression or binding. Concerning putamen, two studies showed no significant change whereas an overexpression of receptors was observed in the other.

**Conclusions:** The hippocampus and amygdala may be pertinent to addictive disorders through their functions on learning and memory, whereas findings in other regions were inconsistent across the studies. Moreover, attention to date has been confined to AUD and heroin/cocaine abuse. Human postmortem studies are prone to degenerative changes after death. These limitations emphasize the need to investigate AMPA receptors in the living human brains.

**Keywords:** Addiction, AMPA Receptors, AMPA Glutamate Receptors, Post-Mortem

**Disclosure:** Nothing to disclose.

### T272. Changes in Calcium Signal Dynamics in the Prelimbic Medial Prefrontal Cortex During Alcohol Consumption in Nondependent and Dependent C57BL/6 J Mice

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**Background:** Alcohol dependence is a chronic relapsing brain disorder characterized by uncontrollable, excessive alcohol consumption. Chronic, heavy alcohol exposure has been shown to alter neural activity in a number of brain regions including, and of

particular interest, the medial prefrontal cortex (mPFC). The mPFC is part of the mesocorticolimbic circuitry, which helps govern responses to and seeking of rewarding stimuli, including alcohol. Additionally, the mPFC is centrally involved in top-down inhibitory control of actions and value-based decision making, and alcohol has been shown to alter the plasticity and excitability of mPFC pyramidal neurons leading to states of hyperexcitability during withdrawal. These changes in mPFC excitability may contribute to the loss of control over alcohol consumption characteristic of dependence. Understanding the dynamics of pyramidal cell signaling in the mPFC during consumption of alcohol prior to, and ultimately after the development of, dependence is vital to understanding how mPFC activity contributes to excessive alcohol consumption.

**Methods:** For the present set of experiments, we used fiber photometry to record calcium activity in the prelimbic mPFC (PL-mPFC) using the genetically encoded calcium indicator, GCaMP6f. GCaMP6f transgene expression was accomplished by infusing the AAV1-CaMKII-GCaMP6f-WPRE viral vector into the PL-mPFC of male C57BL/6 J mice in order to examine calcium activity in putative glutamatergic pyramidal projection neurons. Immediately following viral infusion, a 400 µm multimodal fiber optic probe was implanted just dorsal to the site of virus infusion in the PL-mPFC. Using the TDT Fiber Photometry system and Synapse software, we tested GCaMP signal integrity in an initial cohort of mice ($n = 4$) by briefly anesthetizing the mice with isoflurane and observing a quiescence of the calcium-associated change in fluorescent signal. Additionally, in all subsequent cohorts, GCaMP signaling was verified by observing a robust increase in PL-mPFC calcium activity after a brief startle. In our initial experiment, we examined pyramidal cell activity during consumption of different reinforcing solutions: water, sucrose or alcohol. Calcium transients were recorded in freely moving mice ($n = 10$) allowed access to water (under non-deprived conditions), or 20% (v/v) alcohol, or 1% (w/v) sucrose in their home-cages with lickometers to track consummatory behavior and time-lock GCaMP signaling to bouts of licking. Mice were given 2-hr access to each solution (on separate weeks), 3 h into their dark cycle for 4 consecutive days, and licks and calcium activity were recorded on the 4th day. In a separate cohort of mice ($n = 10$), we sought to determine how alcohol dependence altered activity surrounding bouts for alcohol by recording calcium-dependent GCaMP fluorescence during baseline alcohol consumption, similar to the methods described above, compared to GCaMP signaling during consumption after induction of alcohol dependence. Mice were either rendered dependent on alcohol using the chronic intermittent ethanol (CIE) exposure paradigm, where mice ($n = 5$) were passively exposed to vaporized ethanol for 16 h/day for 4 consecutive days, followed by 72 h of abstinence (1 cycle), or passively exposed to air as a control ($n = 5$). After 4 cycles of CIE (or air) exposure, mice were again given access to alcohol and licks and calcium signaling were again recorded on the 4th day.

**Results:** Calcium signal dynamics were significantly ($p < 0.05$) altered during consumption of alcohol and sucrose (not significantly different from each other) compared to water. Specifically, GCaMP6f signaling increased significantly as mice approached alcohol and sucrose solutions, but not for water. Calcium activity peaked just before initiation of licking, significantly decreased during the consummatory phase, and rebounded back to pre-consumption baseline after the termination of the bout. This phenomenon was more robust for the first bout of alcohol and sucrose compared to the last bout, demonstrating that the magnitude of the signaling changed across the session. Strikingly, induction of alcohol dependence did not alter the pattern of calcium activity surrounding bouts of licking for alcohol, instead, it appears that the prolonged period of no access to alcohol in our air control mice caused a significant reduction in calcium signaling during bouts of licking for alcohol.

**Conclusions:** Consumption of reinforcing solutions, such as alcohol and sucrose, produce similar patterns of calcium-dependent signaling in the PL-mPFC, perhaps encoding some aspect of reinforcing value or salience, as this pattern was absent during consumption of water. Over time, and presumably as alcohol loses some reinforcing value, this signaling diminishes, however alcohol dependence prevents this adaptation in pyramidal cell activity, promoting increased and uncontrolled alcohol consumption. Overall, these results show significant changes in calcium signal dynamics during bouts of licking for rewarding solutions and provide insight into the role of mPFC in consummatory behaviors and demonstrate dysregulation of the PL-mPFC in alcohol dependence. Additionally, these types of studies could be used to validate potential pharmacotherapeutics, such as novel Kv7 channel positive modulators, and determine their ability to to reduce this sustained activation in the PL-mPFC and reregulate these cortical outputs.

**Keywords:** In Vivo Calcium Imaging, Alcohol Dependence, Alcohol Consumption, Ventromedial Prefrontal Cortex, Prelimbic

**Disclosure:** Nothing to disclose.

## T273. Probing Cortical Plasticity in Addiction

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**Background:** Non-invasive brain stimulation has been employed to treat addiction. It also works as a tool for measuring changes in cortical functioning and plasticity. TMS combined EEG allows us to probe cortical dynamics in addicts.

**Methods:** We performed concurrent single-pulse TMS-EEG (spTMS-EEG) before and after 10-minute 10 Hz rTMS. Heroin ($N = 30$) and methamphetamine ($N = 30$) addicts, as well as healthy controls ($N = 30$) were recruited. Each subject received spTMS-EEG at F3, F4, and P3. The EEG data were cleaned using our in-house fully automated artifact rejection algorithm [1]. The spatio-temporal dynamics following the spTMS were then examined. The EEG responses were quantitated using TMS-evoked potentials (TEPs) and event-related spectral perturbations (ERSPs), in both the sensor and source spaces.

**Results:** Following the F3 and F4 stimulation, significant increases of the late ( > 100 ms) TEPs were observed in the post-rTMS sessions compared to the pre-rTMS sessions in the heroin and methamphetamine addicts ($p < 0.05$. Unpaired t-tests on the sensor and source levels, and FDR corrected for multiple comparisons). These changes were identified across a number of key regions for cognitive and emotional processing, including the DLPFC, inferior parietal lobule, and anterior cingulate cortex.

**Conclusions:** Our results reveal for the first time the plasticity changes in addicts induced by the rTMS, which would potentially enable us to assess the treatment efficacy of rTMS.

**Keywords:** Addiction, Cortical Plasticity, Repetitive Transcranial Magnetic Stimulation (rTMS)

**Disclosure:** Nothing to disclose.

**References:**

1. W. Wu, C. Keller, N. Rogasch, P. Longwell, E. Shpigel, C. Rolle, A. Etkin. ARTIST: a fully automated artifact rejection algorithm for single-trial TMS-EEG data. Human Brain Mapping, 39(4), pp. 1607-1625.

## T274. Effects of Chronic Stress Exposure During Withdrawal on the Incubation of Cocaine Craving in Adult Female Rats

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**Background:** Although clinical studies indicate sex differences in both cocaine addiction and stress and anxiety disorders, the majority of preclinical studies on cue- and stress-induced relapse vulnerability have been conducted with male rats. Our own recent studies with adult male rats indicate that chronic stress exposure during early withdrawal from extended access cocaine self-administration accelerates the time-dependent intensification or incubation of cue-induced cocaine craving that occurs during the first few weeks of withdrawal. This enhanced cue-induced seeking behavior observed following chronic stress exposure may make rats more vulnerable to cue-induced relapse during this period. We are currently conducting studies to assess whether similar effects of chronic stress exposure on incubation of cocaine craving are observed in adult female rats and to characterize the time course of these effects.

**Methods:** Similar to our previously published studies with male rats, freely cycling female rats will self-administer cocaine under extended access conditions (6 h per day for 10 days). During the first two weeks of withdrawal, rats will receive repeated restraint stress exposure or control conditions and changes in cue-induced seeking behavior will be assessed at different time points during withdrawal. Prior to all seeking tests, vaginal smears will be performed to determine estrous cycle stage and preliminary analyses will be conducted to determine whether estrous cycle influences stress-induced changes in cocaine seeking behavior.

**Results:** Interestingly, our preliminary results indicate that chronic stress exposure does not accelerate incubation of cue-induced cocaine craving during early withdrawal in female rats ($n = 10$-11 rats/group). Studies are underway to determine whether chronic stress exposure alters cue-induced cocaine seeking at later withdrawal time points.

**Conclusions:** These findings will identify whether sex differences exist in cue- and chronic stress-induced relapse vulnerability and may pave the way for subsequent studies to study the interaction of cocaine and stress in female rats.

**Keywords:** incubation of Cocaine Craving, Chronic Stress, Sex Differences

**Disclosure:** Nothing to disclose.

## T275. Sex-Dependent Influences of Childhood Maltreatment Upon the Neural Correlates of Stress-Induced Cocaine Craving

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**Background:** Childhood maltreatment is a prominent risk factor for the development of substance use disorders. We previously reported the influence of childhood maltreatment upon the neural correlates of stress-induced drug craving in cocaine-dependent men ($n = 38$) (PMC4362751). We expand these previous analyses to a comparison sample of cocaine-dependent women in order to characterize sex differences in how childhood trauma may impact neural processing during stress-induced drug craving.

**Methods:** We recruited 35 adult women [mean(sd) age = 37 (8.0)] meeting DSM-IV criteria for cocaine dependence. Subjects provided informed consent to participate, with approval and oversight by the UAMS Institutional Review Board. Childhood maltreatment was measured via retrospective self-report using the Childhood Trauma Questionnaire (CTQ Total Score). Prior to functional MRI (fMRI) scanning, participants provided personalized scripts describing a stressful event unrelated to drug use. Participants also selected a neutral script describing a nature scene. Scripts were edited to one minute in length and recorded in audio format while read aloud by a female voice in the first person.

Participants underwent fMRI scanning while engaged in mental imagery of each script. Mental imagery consisted of a one-minute period viewing and hearing the script, followed by two minutes of continuing to imagine the event (three minutes total). Each imagery period was followed by a rating period in which subjects indicated (via 10-point Likert scale) their script-induced anxiety, craving to use cocaine, and vividness of mental imagery.

Following standard preprocessing of fMRI data in AFNI, general linear modeling (GLM) estimated changes in brain activity during imagery of stress vs. neutral scripts. Mean beta weights for the GLM contrast of Stress vs. Neutral scripts were extracted for 200 regions of interest (ROIs) comprising a task-based fMRI atlas (PMC4837649). QQ plots of Stress vs. Neutral neural activity indicated heavy tailed distributions (i.e. extreme positive and negative outliers), prompting the use of robust regression with bisquare weighting function implemented in Matlab. Robust regression compared task-dependent neural activity between men and women. Robust regression also related childhood maltreatment, urge to use cocaine, and their interaction effect upon stress-script induced neural activity.

**Results:** Compared to cocaine-dependent men, women showed greater recruitment of the left nucleus accumbens ($t > 4.37$) and bilateral inferior frontal cortex (right $t > 4.20$, left $t > 3.75$) during Stress vs. Neutral imagery. For men, we previously reported an interaction between CTQ and stress-induced craving ratings, with greater scores predicting diminished rostral anterior cingulate during task. This interaction effect was not observed for women. In contrast, women showed an interaction effect for the right posterior dorsolateral prefrontal cortex (DLPFC), with increasing CTQ and stress-induced craving ratings corresponding to increased DLPFC activity. ($t > 4.21$).

**Conclusions:** Sex differences in stress-induced neural activity were observed for nucleus accumbens and lateral inferior frontal cortices, regions which have been respectively associated with deficits of reward processing and response inhibition in substance use disorders. Future work will incorporate clinical and behavioral measures (including drug-use frequency, polydrug use, and subtypes of childhood trauma exposure) to further characterize these reported sex differences in neural activity during stress-induced drug craving.

**Keywords:** Addiction, Stress Models, Childhood Adversity

**Disclosure:** Nothing to disclose.

## T276. Prefrontal and Striatal Contributions to Decision-Making in a Dynamically Updating Risk/Reward Task

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**Background:** Decision making is central to understanding the basic principles of animal behavior. On a continuous basis, animals are tasked with assessing their own motivational state, identifying

options available to increase positive outcomes (such as food), while also correctly identifying costs (such as effort or risk) associated with each available option. As such, decision making represents an integration of competing streams of information. Importantly, choice-related information must be updated regularly in order to maintain optimal decision-making strategies. A significant number of studies have employed relatively sophisticated task designs for rodents in order to understand the neural contributions to decision-making. However, nearly all of these designs employ a relative static choice model in which animals are trained to discriminate between two different options (usually by making a response on the left or right side of the chamber). These designs are typically less sensitive to dynamic changes in risk/reward computations (since the outcomes are stable), and indeed, can make it difficult to identify the specific features of the decision itself which are conflated with other task features (e.g., cues, specific motor responses).

**Methods:** To overcome this limitation, we have adapted a continuous measure of risk/reward judgments from the human clinical literature called the Balloon Analogue Risk Task (BART). In this task, each press on a lever increases the potential payout on that trial. If the rat "cashes out", it will obtain the number of food rewards earned from the press sequence. However, there is a risk the trial will "bust" on each press, at which point the potential rewards earned are lost and the rat must wait for a 30 sec timeout. Thus, on each press, the rat must assess the risks and rewards on each press and update their expectations accordingly. We electrophysiologically recorded in vivo neural activity in the prefrontal cortex (PFC) and either the nucleus accumbens core (NAc; $N = 12$) or dorsal medial striatum (DMS; $N = 3$) of male rats performing the BART task.

**Results:** We found that both cortical and striatal regions contributed to discrete aspects of the task. For example, PFC neurons were preferentially engaged at the moment the animal decided to stop pressing and initiate a cashout response, suggesting an important role in deciding to terminate reward-seeking behavior. Furthermore, we saw robust phasic activity in both PFC and striatal regions at the time of trial busts. To assess a role of dopamine in these processes, rats were given an injection of the selective D3 agonist pramipexole (0.33 mg/kg i.p.) prior to the task on one of the sessions. Relative to a matched session with a vehicle-only saline injection, we found dramatic changes in neural responses in a subset of neurons, particularly during post-response and reward-adjacent epochs that was mirrored in altered behavioral risk-taking profiles.

**Conclusions:** These findings suggest important contributions of corticostriatal circuits in dynamic decision-making behavior, and that dysfunction of these pathways in "hypofrontal" populations (such as following drug abuse or chronic stress) may contribute to these persistent pathological states.

**Keywords:** Risky Decision-Making, Dopamine D3 Receptors, Corticostriatal Circuit

**Disclosure:** Nothing to disclose.

### T277. Involvement of Mesolimbic Endocannabinoid Signaling in Stress-Induced Escalation of Cocaine Intake in Rats

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**Background:** Clinical evidence has identified stress as an important contributing factor to substance use disorder (SUD). This is particularly problematic as stress is unavoidable in daily life. Therefore, understanding the neurobiological mechanisms that underlie the contribution of stress to SUD is critical. One characteristic of SUD is a loss of control over drug intake that is modeled, in the rat, by conditions that result in escalating patterns of drug self-administration (SA). Repeated daily stress at the time of SA induces an escalation of cocaine intake in a glucocorticoid-dependent manner. This stress-induced escalation of SA likely involves neurobiological mediators that connect stress-responsive and reward systems in the brain, such as the endocannabinoid system (eCB), in regions implicated in both stress and reward, such as the nucleus accumbens shell (NAc) and ventral tegmental area (VTA). We hypothesize that repeated stress at the time of SA induces a persistent increase in eCB signaling, particularly in the NAc shell and VTA, that results in escalation of cocaine use and increased susceptibility to later reinstatement.

**Methods:** Male SD rats were trained to SA cocaine (0.5 mg/kg/inf) on a FR 4 schedule in $4 \times 30$ min SA sessions separated by 5-min drug-free periods. Some rats received intermittent electric footshock stress in the SA chamber during the 5 min drug-free period over 14 days. Rats were tested for the persistence of elevated cocaine intake in the absence of the stressor and increased susceptibility for reinstatement to various stimuli. We examined the involvement of endocannabinoid signaling in stress-escalated cocaine intake by administration of the cannabinoid receptor type 1 (CB1R) antagonist AM251 systemically (0, 1 mg/kg, i.p.) or directly into the NAc shell or VTA (0, 1, 3 µg) prior to a SA session. Changes in CB1R binding and density are being examined in the NAc shell and VTA following SA under stress and non-stress conditions. Lastly, AM251 (1 mg/kg, i.p.) was administered prior to cocaine-primed reinstatement (10 mg/kg, i.p.) to test for involvement of endocannabinoid signaling in augmented reinstatement following stress-induced escalation of cocaine intake.

**Results:** Electric footshock stress administered daily at the time of self-administration induced an emergent escalation of cocaine intake over 14 days that persists in the absence of stress ($n = 13$-15/group, $p < .05$). Stress-escalated rats also demonstrate increased susceptibility to later reinstatement. Rats with a history of stress at the time of SA show augmented reinstatement to a priming injection of cocaine (2.5, 5, 10 mg.kg, i.p.; $n = 11$-14/group, $p < .05$), re-introduction of the footshock stress during the 5-min drug-free period ($n = 9$-12/group), and to an injection of the alpha-2 adrenergic receptor antagonist yohimbine (0, 1.25, 2.5 mg/kg, i.p.; $n = 7$-8/group, $p < .05$). Systemic administration of AM251 prior to SA attenuates cocaine intake only in stress-escalated rats ($n = 7$-8/group, $p < .05$). We have localized this effect to the NAc shell and VTA as direct administration of AM251 into either region prior to SA attenuates cocaine intake only in stress-escalated rats ($n = 6$-7/group, $p < .05$). Lastly, systemic administration of AM251 prior to reinstatement attenuates cocaine-primed reinstatement only in rats with a prior history of stress at the time of SA ($n = 8$-9/group, $p < .05$).

**Conclusions:** Chronic stress induces a glucocorticoid-dependent escalation of cocaine intake that is the result of persistent neuroadaptations. These neuroadaptations likely result in long-lasting changes in the endocannabinoid system as repeated stress recruits endocannabinoid signaling in the NAc shell and VTA to drive drug use. Additionally, these stress-induced neuroadaptations are long-lasting and likely occur in regions critical for drug-seeking behavior. Understanding the unique mechanisms by which stress can drive drug use has implications for identifying and treating sub-populations of addicts in whom stress is a contributing factor.

**Keywords:** Chronic Stress, Endocannabinoids, Glucocorticoids, Cocaine Self-Administration, Ventral Tegmental Area (VTA)

**Disclosure:** Nothing to disclose.

### T278. Chemogenetic Ventral Tegmental Area Dopamine Stimulation Reveals Persistent Prefrontal Cortex Endocannabinoid Disruption After Adolescent Cannabinoid Microdosing

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**Background:** Adolescence is a critical window of maturation for reward-related brain circuits, which is in part orchestrated by endocannabinoid (ECB) signaling. Exogenous perturbation of cannabinoid transmission with drugs like cannabis during adolescence may therefore have consequences on mesolimbic reward circuit development, and persistently alter motivated behavior into adulthood. ECB signaling regulates ventral tegmental area (VTA) dopamine (DA) projections to forebrain regions like nucleus accumbens (NAc) and dorsal medial prefrontal cortex (dmPFC), yet it remains unclear how exogenous cannabinoid receptor stimulation during adolescence alters DA circuits, and DA-dependent behaviors. In addition, most adolescent exposure studies to date (including ours) have used moderate to high doses of cannabinoid drugs, which can produce anxiogenic states or other nonselective effects when administered to adolescent animals. Low doses of cannabinoid drugs, which are reinforcing in adolescents, have rarely been tested. Therefore, we employ an adolescent microdosing protocol (1/10th that used in our previously published report (Schoch et al., 2018)), and examine long-lasting effects on mesocorticolimbic DA circuits and motivated behaviors.

**Methods:** Here, we dissect DA system function in adult female and male tyrosine hydroxylase:Cre (TH:Cre) rats following adolescent cannabinoid microdosing, using DA neuron-specific chemogenetic activation to recruit endocannabinoid transmission, and measure outcomes on Fos expression, endocannabinoid level analysis with mass spectrometry/liquid chromatography, and behavioral analyses. TH:Cre rats were treated with a "microdose" of the synthetic CB1/2 agonist, WIN 55,212-2 (0.12 mg/kg; Adol-WINμ), or vehicle (Adol-Veh) daily during postnatal days 30-43, corresponding to early- to mid-adolescence. Then, Cre-dependent excitatory (hM3Dq) designer receptors exclusively activated by designer drugs (DREADDs) were bilaterally expressed in VTA DA neurons (or not expressed in Cre-negative littermate controls), thereby allowing precise control of DA neurons upon systemic administration of the DREADD agonist, clozapine-N-oxide (CNO; 10 mg/kg). Rats were trained to self-administer palatable banana-flavored pellets and a contingent light + tone cue for 10 days, then underwent two consecutive extinction training sessions on CNO. Additional drug-free extinction training was then conducted until criterion was met ( < 25 active lever presses/day) followed by two cue-induced reinstatement tests (CNO and vehicle, counterbalanced). Wildtype and hM3Dq-expressing Adol-WINμ and Adol-Veh rats also underwent locomotor activity tests following CNO, after which they were sacrificed for analysis of Fos in NAc and dmPFC. Separate groups of rats were also tested to determine: 1) ECB levels in dmPFC and NAc following VTA DA neuron stimulation, 2) whether dmPFC ECB transmission is necessary for potentiated VTA DA-stimulation of behavior, and 3) to verify that WINμ is reinforcing and non-anxiogenic.

**Results:** Chemogenetically stimulating VTA DA neurons in Adol-WINμ rats potentiated DA stimulation-induced reward seeking behaviors (relative to Adol-Veh rats; $p < 0.05$), and altered neural activity (Fos) and ECB response to DA neuron manipulation ($p < 0.01$). We observed no overt effects on behavior or neural outcomes in the absence of DA stimulation (or with CNO in the absence of DREADDs), demonstrating that effects are contingent upon strong circuit activity, here induced chemogenetically. We also re-created in control rats the behavioral phenotype of Adol-WINμ rats (potentiated locomotor response to DA neuron stimulation), by mimicking the dmPFC ECB signaling environment pharmacologically ($p < 0.05$), showing that dmPFC ECB changes seen after Adol-WINμ are causal of potentiated behavioral responses.

**Conclusions:** Our results reveal that adolescence is a critical window of development during which even small doses of cannabinoids drugs can persistently alter mesolimbic DA circuit neural and endocannabinoid activity, causing potentiated reward-seeking behaviors that could have implications for adulthood responses to addictive drugs, stress, or other factors that recruit VTA DA neurons.

**Keywords:** Chemogenetics, Self-administration, Cannabinoids

**Disclosure:** Nothing to disclose.

### T279. Interactions Between Ceftriaxone and Instrumental Extinction Training on the Circuitry Underlying Cued Relapse to Cocaine Seeking

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**Background:** Cocaine use disorder is characterized by compulsive drug use and the rate of relapse is high. Drug-associated cues and contexts maintain conditioned reinforcing properties that trigger relapse. Our lab uses a rat model of intravenous cocaine self-administration and relapse and has found that impaired glutamate homeostasis mediates relapse to cocaine-seeking. Ceftriaxone, a β-lactam antibiotic, restores glutamate homeostasis that is impaired by chronic cocaine self-administration. Subchronic treatment with ceftriaxone reduces cocaine-seeking during cocaine- and cue-primed tests of relapse following instrumental extinction training. Glutamate release in the nucleus accumbens (NA) core drives reinstatement and ceftriaxone prevents this release during cocaine-primed reinstatement after instrumental extinction. However, we have shown that in the absence of instrumental extinction, ceftriaxone is unable to prevent glutamate efflux in the NA core during context-primed relapse to cocaine-seeking. Post-drug experience (abstinence vs. extinction) alters glutamatergic adaptations in the NA after cocaine. Here, we investigated whether extinction training is required for ceftriaxone to prevent glutamate efflux when cocaine-associated cues are used to prime relapse. We also assessed Fos expression in regions known to mediate cocaine relapse to test the hypothesis that ceftriaxone would attenuate neuronal activation throughout this circuitry in a different manner following abstinence and extinction.

**Methods:** Male rats underwent intravenous cocaine self-administration during which time cocaine infusions were accompanied by discrete drug-associated cues (light + tone). Rats then experienced 21 days of either instrumental extinction training or abstinence in the homecage. A subset of rats in each condition were administered ceftriaxone (200 mg/kg IP) for 6 days prior to a cue-primed reinstatement test, while the remaining rats received vehicle. Rats were tested for cue-primed reinstatement during a microdialysis session with probes inserted into the nucleus accumbens (NA) core. Glutamate content in microdialysis samples was quantified with HPLC. Rats were perfused immediately following the test, brains sliced and stained for Fos immunoreactivity in regions sending glutamate projections to the NA core.

**Results:** We found that following both abstinence and instrumental extinction training, ceftriaxone reduced cue-primed cocaine relapse and the associated glutamate efflux in the NA

core. After abstinence, but not extinction, rats treated with ceftriaxone displayed increased Fos expression in the infralimbic cortex that negatively correlated with lever pressing during reinstatement. After abstinence, but not extinction, ceftriaxone-treated rats displayed reduced Fos expression in the VTA and BLA.

**Conclusions:** These data support the ability of ceftriaxone to attenuate relapse and glutamate efflux in the NA core in the absence of extinction training but indicate that ceftriaxone interacts with a different neuronal circuitry depending on whether abstinence or instrumental extinction is experienced.

**Keywords:** Glutamate Homeostasis, GLT-1, Prefrontal Cortex, Amygdala, VTA

**Disclosure:** Nothing to disclose.

### T280. Regulation of Compulsive-Like Alcohol Seeking in Adult Male Mice via Accumbens mGluR2/3 Agonism

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**Background:** The ability to flexibly regulate behavior is impaired in many neuropsychiatric illnesses, including alcohol use disorders. The performance of compulsive-like reward seeking that occurs despite adverse consequence may result from an imbalance of approach and avoidance behaviors. Flexible behavior is thought to be in part regulated by glutamate signaling within limbic corticostriatal circuits, and alcohol use disorders are associated with a hyperglutamatergic state within the nucleus accumbens. We have previously observed that regulation of glutamate signaling within the nucleus accumbens shell modulates response strategy selection, suggesting that glutamatergic function within this structure may regulate flexible reward seeking. Here, we assessed whether modulation of glutamate signaling via mGluR2/3 agonism in the nucleus accumbens shell and additional striatal subregions regulate the expression of compulsive-like alcohol seeking.

**Methods:** To investigate subregion specific effects of mGluR2/3 agonism, adult male c57BL6 mice underwent cannulation targeting either the nucleus accumbens shell, nucleus accumbens core, or the dorsolateral striatum (n's = 6-12). These experiments were approved by the Drexel University College of Medicine IACUC and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals. After recovery, mice will be trained in a modified conditioned place preference (CPP) paradigm in which they are conditioned to associate one context with ethanol reward. Conditioning chambers are standard three chamber boxes with distinct walls (vertical black and white stripes or diagonal marble and black stripes) and floors (wire mesh or grid). The two conditioning chambers are separated by a neutral, gray chamber. During 3 conditioning sessions, mice will be confined for 20 min to a "paired" chamber immediately after an i.p. injection of 2 g/kg ethanol in saline, a dose we have shown to produce CPP in mice. On 3 alternating days, mice will be restricted to the "unpaired" chamber and will receive i.p. injections of saline immediately prior to placement. On day 7, mice will be placed in the center chamber and allowed to freely explore all chambers for 5 min, and latency to enter each chamber will be measured. This time point enables examination of latency to enter both chambers but minimizes extinction learning. On day 8, mice will be confined to the reward-paired chamber. One minute after placement in the chamber, mice will receive a 2 sec, 0.8 mA shock, and remain in the chamber for 60 seconds after the shock. To test compulsive-like ethanol seeking, mice will be returned to the gray chamber on day 9, and latency to enter the EtOH chamber will be assessed. Mice will be matched by locomotor behavior after EtOH administration and by preference scores during a pre-conditioning session to receive either LY379269 (1ug/side in 0.2ul sterile saline), an mGluR2/3 agonist, or saline vehicle into either the shell, core, or dorsolateral striatum 30 min prior to test on days 7 and 9. The effect of systemic mGluR2/3 agonism on compulsive-like reward seeking was assessed in a separate cohort of animals. Training parameters were identical except that LY379268 or saline was administered i.p.(3 mg/kg) 30 min prior to test.

**Results:** Data were analyzed by rmANOVA as appropriate. Our findings indicate that local administration of an mGluR2/3 agonist within the nucleus accumbens shell reduces ethanol-reward seeking following an adverse consequence without impacting ethanol CPP. LY379268 administration in the shell resulted in a greater reduction in time spent in the reward paired chamber after pairing that chamber with an adverse consequence (footshock) ($p < 0.05$). In contrast, administration of an mGluR2/3 agonist within the core or the dorsolateral striatum did not impact either the expression of ethanol CPP or compulsive-like ethanol seeking following a footshock (p's > 0.05). Of note, systemic administration of an mGluR2/3 agonist did not reduce compulsive-like ethanol seeking, but rather mice receiving LY379268 systemically were further resistant to the effects of an aversive stimuli on ethanol seeking ($p < 0.05$). Specifically, systemic LY379268 administration reduced sensitivity to the effects of footshock on time spent in the ethanol chamber, suggesting that systemic effects were mediated by structures other than the nucleus accumbens shell.

**Conclusions:** Together these data identify a role for glutamate signaling within the nucleus accumbens shell in a model of compulsive-like alcohol seeking. Specifically, we observe that local administration of LY379268 within the shell results in a greater reduction in time spent in an alcohol reward-paired chamber following a footshock. mGluR2/3 agonism within the nucleus accumbens core and dorsolateral striatum did not impact compulsive-like ethanol seeking. Of note, we observed an opposing effect of systemic administration of LY379268 on behavior such that systemic mGluR2/3 agonism enhanced compulsive-like reward seeking. Together these data suggest that regulation of glutamate signaling specifically within the nucleus accumbens shell can regulate compulsive-like ethanol seeking when conflict between ethanol reward and an aversive stimulus is present. Ongoing and future experiments are determining the glutamatergic projections to the shell that contribute to this effect.

**Keywords:** Metabotropic Glutamate Receptor 2 (mGluR2), Nucleus Accumbens Shell, Striatum, Alcohol and Substance Use Disorders

**Disclosure:** Nothing to disclose.

### T281. Loss of an Evolutionarily-New Form of Glutamate Release Alters Cognition While Preserving Basic Brain Function

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**Background:** Impaired cognition underlies a range of psychiatric disorders such as drug addiction which renders identifying its molecular basis as a high scientific priority. Aberrant glutamate signaling is a common pathological factor in cognitive impairment across distinct CNS disorders. However, the ubiquitous nature of glutamate poses a critical challenge in establishing the pathological basis of impaired cognition in some CNS diseases in which it is necessary to identify genes that, when disrupted, impair

relevant forms of cognition without broadly altering brain function that preserved in these disorders. This is illustrated by observations that genetic models impairing synaptic glutamate produce widespread, fatal disruption of brain function. Notably, while synaptic glutamate neurotransmission has been present since the emergence of nervous systems, this ancient form of intercellular signaling became enriched through the accumulation of evolutionarily-new genes. As a result, glutamate signaling in vertebrates involves a network of glutamate receptors, transporters, and release mechanisms expressed by neurons, astrocytes, and other cells in the brain. Hence, there is a need to determine whether the molecular basis of pathological glutamate signaling involves evolutionarily-new genes that encode non-synaptic, intercellular signaling. Here, we examined in rat the functional relevance of the evolutionarily-new gene Slc7a11, which encodes for the catalytic subunit (xCT) of the nonvesicular glutamate release mechanism system xc- (Sxc).

**Methods:** A phylogenetic tree for xCT was constructed from publically available sequences of vertebrate species. Amino acids under selective pressure were determined using a SLAC analysis. Rats lacking functional Sxc (MSxc rat) were developed by mutating the Slc7A11 gene and used for molecular and behavioral experiments. Astrocyte cell cultures were generated from PN day 3 rats and used to assess Sxc activity. 3H-D-aspartate uptake was measured to determine EAAT function. Western blotting was used to measure glutamate-related proteins. A novel transgenic rat was created using a transgene enabling GFAP promoter-driven eGFP expression. This rat line was used for fluorescence activated cell sorting (FACS). Sorted astrocytes were analyzed for gene expression using RT-PCR. Whole-cell patch-clamp recordings were obtained from distinct nucleus accumbens core (NAcc) efferent projections which were labeled by injecting retrograde fluorescent tracers into the ventral pallidum (VP) or the substantia nigra (SN). Behavioral experiments included attentional set shifting, a Kamin fear blocking paradigm, and simple associative learning tasks e.g. lever press for sucrose. Physiological telemetry included locomotor activity, core body temperature, and mass gain.

**Results:** xCT is expressed by astrocytes within the NAcc ($n = x$), phylogenetically conserved, and evolved under significant negative selective pressure in vertebrate species ($n = 115$). Mutation of Slc7a11 produced a global knockout of the protein which led to disruptions in astrocytic EAAT function ($N = 15$-$18$/genotype; $t31 = 2.69$, $p < .05$) as well as an enhancement in GluA1 phosphorylation at the T840 residue ($N = 7$-$8$/genotype; $t13 = 2.93$, $p < .05$) within the NAcc. Remarkably, MSxc rats had enhanced mEPSC frequency ($t26 = 2.7$, $p < .05$) and amplitude ($t26 = 2.35$, $p < .05$) within the NAcc to SN pathway ($N = 14$/genotype) in the absence of altered activity in NAcc to VP projections ($n = 14$/genotype; $p > 0.05$). Next, we found that eliminating Sxc-mediated glutamate release impaired behaviors requiring complex cognition without altering more ancestral, essential forms of brain function. Specifically, we did not observe a genotypic effect when measuring growth rates, activity states, central regulation of metabolism, activity in a novel object recognition task, or in the formation of simple associations ($N = 7$-$31$/genotype; $p > 0.05$). However, MSxc rats displayed impaired gating of associative processes in the Kamin blocking paradigm ($N = 8$/genotype; Tukey post hoc, $p < 0.05$) and cognitive flexibility in a maze-based attention set-shifting task ($N =$ /genotype, Tukey HSD, $p < 0.05$).

**Conclusions:** These findings reveal the existence of an evolutionarily-new gene expressed by astrocytes that is selectively involved in complex cognition. Hence, understanding the evolution of nervous systems may help address the challenge of identifying genes that could underlie impaired cognition while preserving other domains of brain function. Moreover, a loss of Sxc mediated signaling between astrocytes and neurons produces pathway-specific changes in neural circuits in a manner that impacts higher-order processing.

**Keywords:** Glutamate Homeostasis, Astrocytes, Cognition

**Disclosure:** Prementis Pharmaceuticals, Consultant

## T282. Chemogenetic Modulation of Direct and Indirect Pathway Striatal Medium Spiny Neurons Alters Drug-Seeking in a Rat Model of Heroin Addiction

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**Background:** Opioid addiction, which is marked by cycles of compulsive drug-taking and drug-seeking and high rates of relapse, has reached epidemic proportions. Although susceptibility to addiction varies, overdose deaths are at unprecedented levels, yet are but one of the many escalating costs of this disease. Disambiguating the complex neurobiological changes that regulate pathological opioid use in vulnerable individuals is therefore critical for curtailing this national crisis. The striatum has been identified as a key relay site within the cortico-basal ganglia-thalamic circuitry that drives addiction. However, the primary output neurons of the striatum (i.e., GABAergic medium spiny projection neurons (MSNs)), guide behavioral output through two divergent pathways (the direct and the indirect). Although opioid administration has been shown to induce an array of cellular and molecular changes in both direct pathway MSNS (dMSNs) and indirect pathway MSNs (iMSNs) across striatal regions, the functional role of these cell populations in the development of patterns of heroin-taking and heroin-seeking that are characteristic of human addiction is unknown.

**Methods:** To begin to address question, we used viral expression of Gi/o- and Gq-DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) to transiently modulate the activity of dMSNs and iMSNs during heroin-taking and -seeking behaviors in rats expressing low and high levels of addiction-like behavior following heroin use. DREADDs were expressed using a combinatorial viral vector approach, using infusions of a retrograde CAV-2-Cre virus into either the ventral tegmental area (VTA) or ventral pallidum (VP) along with infusion of an AAV-DIO-hM4Di or AAV-DIO-hM3Dq virus into the NAc to allow pathway-specific manipulation of dMSNs and iMSNs, respectively. Rats were trained to self-administer heroin (0.075 mg/kg/infusion) on an FR1 schedule using an intermittent-access paradigm (5 min ON, 25 min OFF; 6 h total) for 10 days, during which drug delivery was signaled by a cue light. Rats then underwent a progressive ratio (PR) test for motivation and additional baseline sessions, followed by extinction training and a cue-induced reinstatement test. Rats received injections of vehicle or clozapine-N-oxide (CNO; 5 mg/kg) prior to the PR test and cue-induced reinstatement.

**Results:** We found that chemogenetic activation of dMSNs enhanced cue-induced reinstatement in rats expressing a high (but not low) addiction-severity phenotype following heroin use whereas chemogenetic inhibition of these neurons had the opposite effect. In contrast, chemogenetic activation of iMSNs decreased cue-induced reinstatement in all rats (i.e., rats expressing either a high or a low addiction-severity phenotype) whereas inhibition of these neurons had the opposite effect. Unexpectedly, these manipulations had no effect on motivation to take heroin, using a PR test.

**Conclusions:** These results suggest that both dMSNs and iMSNs in the NAc modulate heroin-seeking, but in opposing ways. In order to extend these findings, calcium imaging experiments

using in vivo fiber photometry are currently underway to determine how activity of dMSNs and iMSNs mark cues associated with heroin use and heroin-seeking.

**Keywords:** Heroin Self-Administration, Drug Addiction, Nucleus Accumbens

**Disclosure:** Nothing to disclose.

### T283. A Novel Hypothalamic-To-Thalamic Circuitry Controlling Motivated Nicotine Seeking

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**Background:** Motivation can be broadly defined as a process whereby internal states and external stimuli invigorate and hone on-going behavior. Theories of the etiology of neuropsychiatric disorders such as depression, schizophrenia and drug abuse have integrated aberrant control of reward, motivation, and salience attribution as drivers of such disease symptomology. The mesolimbic dopamine system is a key node in the neural circuitry underlying motivation, and thus a likely site of dysregulation in across disorders that feature motivational deficits. Orexin (OX; hypocretin) neurons of the lateral hypothalamus (LH) can modulate activity in the mesolimbic dopamine system and are thus positioned to have a major influence on both normal and pathological motivated behaviors. Indeed, the orexin system has specifically been implicated in modulating motivation to seek drug rewards including psychostimulants, opiates, and nicotine; though the precise circuitry that allows the orexin system to contribute to such motivational control generally and drug reward specifically is still being defined.

**Methods:** In the present set of studies, we set out to identify the circuits and mechanisms of OX modulation of the motivational properties of nicotine. To this end, male and female rats and mice ($n = 9$-15 per study) were trained in nicotine self-administration. The capacity of orexin to modulate nicotine self-administration by was assessed using a combination of behavioral pharmacology, genetic deletion/re-expression of the OX-1 receptor, inhibition/excitation of defined OX-circuits with DREADDS, immunohistochemistry, and $Ca+2$-imagning with fiber photometry from OX cell bodies in the lateral hypothalamus during nicotine self-administration. Additional experiments employed slice electrophysiology to interrogate the local synaptic mechanisms underlying OX control of nicotine seeking behavior. All studies were conducted in accordance with the procedures outlined in National Institutes of Health Guide for the Care and Use of Laboratory Animals, as well as the Mt. Sinai IACUC.

**Results:** We found that rates of nicotine self-administration are markedly decreased, in a gene-dosage dependent manner, in mice with one or two null alleles, respectively, of the Hcrtr1 gene, which encodes the orexin-1 receptor (OX1R). Strikingly, when the work (lever pressing) required to obtain nicotine was decreased, accomplished by reducing the reinforcement schedule from a fixed ratio 5 (FR5) to a FR1, deficits in intake were completely reversed in the mutant mice. This suggests that orexin transmission specifically regulates the motivation to obtain nicotine under effortful conditions, but not the reward-related actions of the drug that support its consumption. Accordingly, recordings of calcium dynamics in OX cell bodies during self-administration revealed that OX neural activity specifically coded the effort associated with obtaining nicotine, but not nicotine receipt. Orexin is thought to modulate the behaviorally reinforcing properties of addictive drugs by direct stimulatory actions on dopamine neurons in the ventral tegmental area (VTA). However, we found that virus-mediated re-expression of the Hcrt1 gene in the VTA of the knockout (KO) had no impact on their deficits in nicotine self-administration. Similarly, local infusion of a selective OX1R antagonist into the VTA did not reduce nicotine responding in rats. To identify brain circuits that explain this deficit in OX1R mutant mice, we assessed brain c-Fos levels after a nicotine injection in wild-type (WT) and Hcrtr1 KO mice. We found a small pocket of cells in the dorsal lateral thalamus (DLT) that were more robustly activated in KO mice than WT mice. Using a Hcrtr1-eGFP reporter line of mice, as well as GAD2-Cre mice, we identified a small population of OX1R-expressing cells in this same part of the DLT, located immediately adjacent to the habenula, that are GABAergic. Virus-mediated re-expression of the Hcrtr1 gene in the DLT of Hcrtr1 KO mice completely restored their nicotine responding under a FR5 schedule. Conversely, infusion of an OX1R antagonist into DLT, or inhibition of $GAD2+$ cell bodies in the DLT, decreased nicotine responding in rats and mice tested under a FR5 but not FR1 schedule.

**Conclusions:** Together, these results suggest that in contrast to psychostimulants like cocaine, or opiates, the circuitry underlying the ability of OX-signaling to modulate nicotine intake lies outside the canonical VTA-mesolimbic pathway. Specifically, LH OX projections regulate the effortful seeking of nicotine by controlling local inhibitory transmission in the DLT via OX1Rs. The surprising discovery of a dedicated circuitry for modulating effortful nicotine seeking suggests that more broadly, altered OX modulation of this novel hypothalamic-- > thalamic circuit may represent a locus of dysfunction in addiction as well as other neuropsychiatric illnesses in which deficits in motivation are a core symptom. The results of these studies provide new insights into the neural circuitry of motivation and may identify important opportunities for new therapeutic development for the treatment of motivational deficits across neuropsychiatric conditions.

**Keywords:** Nicotine Addiction, Orexin System, Thalamus

**Disclosure:** Nothing to disclose.

### T284. Induction of Long-Term Depression at Medial Geniculate Thalamic Synapses in the Lateral Amygdala Blocks Cue-Induced Cocaine Seeking and Mimics the Effects of Cue Extinction

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**Background:** Cocaine use disorder causes significant health and financial burdens to society; however, no effective treatments currently exist. One of the biggest hurdles for successful treatment is the high likelihood of relapse in abstinent individuals. Relapse is often triggered by exposure to the environmental stimuli or cues that were associated with prior cocaine use. Thus, a potential treatment strategy is to reduce the strength of cocaine-associated memories, thus reducing the likelihood of relapse. One such strategy, exposure therapy, involves a process known as memory extinction, where cues are presented repeatedly in the absence of drug reinforcement. Unfortunately, clinical application of exposure therapy has met with limited success. Thus, an increased understanding of the basic neurobiological processes underlying cocaine memory encoding and its extinction is needed to develop improved memory-based treatments for addiction. Evidence from studies of fear conditioning suggest that the formation of emotionally valenced memories involves the strengthening of synapses onto lateral amygdala (LA) neurons receiving input from sensory thalamus, and that extinction can weaken these

synapses. Thus, we hypothesized that cocaine memories may also be encoded in this circuit and that manipulation of the strength of these synapses would reduce cue-induced cocaine seeking.

**Methods:** Male Sprague-Dawley rats were infused with a virus expressing the channel rhodopsin variant oChIEF into the medial geniculate nucleus of the thalamus (MGN) and were trained to self-administer intravenous cocaine or saline paired with an audiovisual cue. In experiment 1, rats were then divided into groups that either underwent cocaine cue extinction (120 unreinforced cue presentations) or a control procedure (placement in box with no cues presented). The following day brain slices of the LA were prepared for electrophysiological recordings. MGN-LA synapses were stimulated by blue light activation of oChIEF and post-synaptic EPSCs were recorded. In a subset of animals, we measured the induction of long-term depression (LTD) between groups using 900 2-ms pulses of 473-nm light, at 1 Hz over 15 min. Experiment 2 was similar to experiment 1, except that rather than cue extinction, rats underwent in vivo optogenetic LTD induction or sham stimulation and were tested for cue-induced reinstatement of cocaine seeking 24 h later.

**Results:** Experiment 1: Rats that self-administered cocaine exhibited significantly larger EPSC amplitudes at MGN-LA synapses relative to saline controls. Rats that underwent cue extinction did not exhibit synaptic potentiation, having EPSC amplitudes that were not significantly different from saline controls (One-way ANOVA $F(2,25) = 6.87$, $P = .004$). In addition, while LTD could be induced at MGN-LA synapses in the cocaine group (Paired t-test, $t(6) = 3.34$, $^*P = .016$), LTD was not able to be observed in saline trained or extinction trained rats, suggesting that extinction depotentiates these synapses occluding further LTD. Experiment 2: In vivo LTD at MGN-LA synapses produced a robust and sustained reduction in cue-induced reinstatement relative to sham stimulated controls (Two-way ANOVA, main effect of group ($F(1,11) = 18.16$, $P = .001$) and a day x group interaction ($F(1,11) = 12.76$, $P = .004$); post hoc analysis: $^{**}p < .01$.)

**Conclusions:** These results suggest that cocaine cue memories are at least partially encoded at MGN-LA synapses and the extinction of these memories reduces the strength of these synapses. Moreover, our results suggest that stimulation of these synapses in a manner that induces LTD may be a method for reducing the ability of cocaine cues to induce relapse in a more effective manner than current behavioral therapies.

**Keywords:** Cocaine Self-Administration and Reinstatement, Extinction Learning, Amygdala, Medial Geniculate Nucleus, Long Term Depression

**Disclosure:** Nothing to disclose.

### T285. Ventral Striatal Oscillations Identify Periods of Addictive Substance Consumption

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**Background:** Addiction-related disorders produce increased rates of morbidity and mortality and complicate the management of other psychiatric conditions. The brain networks underlying the consumption of addictive substances, e.g., highly palatable food or alcohol, have a nexus point within the ventral striatum (VS). The VS continues to be evaluated as a target for circuit-based interventions (e.g., deep brain stimulation) in depression, obsessive compulsive disorder, and addiction-related disorders. Emerging evidence suggests that dynamic changes in local field potential (LFP) oscillations recorded in the VS are related to underlying neuronal processes. If relevant information could be extracted from these oscillations in real-time from stimulating electrodes, then it could be used in closed-loop and adaptive neuromodulation systems to individually tailor treatments to improve outcomes. We have previously presented data demonstrating that VS LFPs can identify periods of time when rats are consuming a high-sugar/high-fat (HS/HF) diet versus all other behaviors. Here, we tested the hypothesis that VS LFP oscillations could be used to identify periods of alcohol consumption in real-time. Since patients with addiction often use several substances, we determined if the models that identified periods of alcohol consumption could also identify periods of HS/HF diet consumption and vice versa. Last, we determined if models built from combined (alcohol and HS/HF diet) datasets would be better able to identify the consumption of either alcohol or HS/HF diet compared to the single substance models.

**Methods:** Male and female Sprague-Dawley rats were implanted with electrodes targeting the VS (nucleus accumbens shell [NAc]) bilaterally and local field potentials with video were recorded during limited access sessions to 10% alcohol (as previously with HS/HF diet). Videos were analyzed offline to identify relevant behavioral intervals (e.g., drinking or feeding) (Plexon system). Custom code written in Matlab was used to extract LFP features of power and phase coherence across 6 established frequency bands (delta, theta, alpha, beta, low gamma and high gamma) from 1-90 Hz. The LFP features were then used as predictors in logistic regression models to classify rat behavior (consuming alcohol vs. all other behaviors) in real time (using 5-second analysis windows). Twenty different model versions were built using random selections of 80% of the data that were then evaluated using the corresponding data not used for model building (20%). Models built to identify periods of alcohol consumption were also tested on data from another cohort of animals conditioned to binge a HS/HF diet and vice versa. Last, generalized models were built from both the alcohol ($N = 10$) and HS/HF diet ($N = 12$) datasets to identify periods of consumption of either substance versus all other behaviors. To determine if models were significantly outperforming chance, all analyses were performed on permutations of the data using Monte Carlo sampling and then the two distributions (real vs. permuted) of model performances (area under the receiver operator characteristic curves [AUC]) were described with ($\pm 95\%$) confidence intervals. If models built from all LFP features outperformed the permuted models, then an exhaustive evaluation of each LFP feature (logistic regression) was carried out to determine the information attributable to each feature.

**Results:** LFP features extracted from NAc recordings during limited access to alcohol identified 5-second periods of LFP data as belonging to periods when the rat was consuming alcohol versus all other behaviors with an average $AUC = 0.91 \pm 0.016$. The models that were built to identify periods of alcohol consumption performed worse than chance ($AUC \approx 0.5$) when identifying periods of HS/HF diet consumption (average $AUC = 0.36 \pm 0.0082$). Likewise, models built to identify periods of HS/HF diet consumption versus all other behaviors ($AUC = 0.79 \pm 0.006$) also performed worse than chance when identifying periods of alcohol consumption (average $AUC = 0.3 \pm 0.028$). However, when data from both the HS/HF diet and alcohol studies were combined to build models that could identify periods of alcohol or HS/HF diet consumption versus all other behaviors—performance exceeded chance ($AUC = 0.72 \pm 0.036$). When these generalized models were evaluated on either alcohol or HS/HF diet alone, it did better on the alcohol data ($AUC = 0.82 \pm 0.069$) than the HS/HF diet data ($AUC = 0.65 \pm 0.039$).

**Conclusions:** These findings demonstrate that periods of either alcohol or HS/HF diet consumption can be distinguished from all other behaviors using ventral striatal oscillations. The substance specific models, however, identified periods of consumption more

accurately then the generalized models that worked for either substance—highlighting the performance trade-offs.

This work indicates VS oscillations can be used to identify periods of consumption of at least two distinct rewarding substances from other behaviors. The demonstration that brain oscillations can be used to determine when addiction-related behavior is occurring is a critical step in the development of next generation closed-loop and adaptive neuromodulation systems for the treatment of addiction-related disorders.

**Keywords:** Local Field Potentials, Nucleus Accumbens, Alcohol, Food Addiction

**Disclosure:** Nothing to disclose.